Characterization of PCF Power Station Combustion Wastes Using Total Organic Halide Analysis and Toxicity Screening Tests

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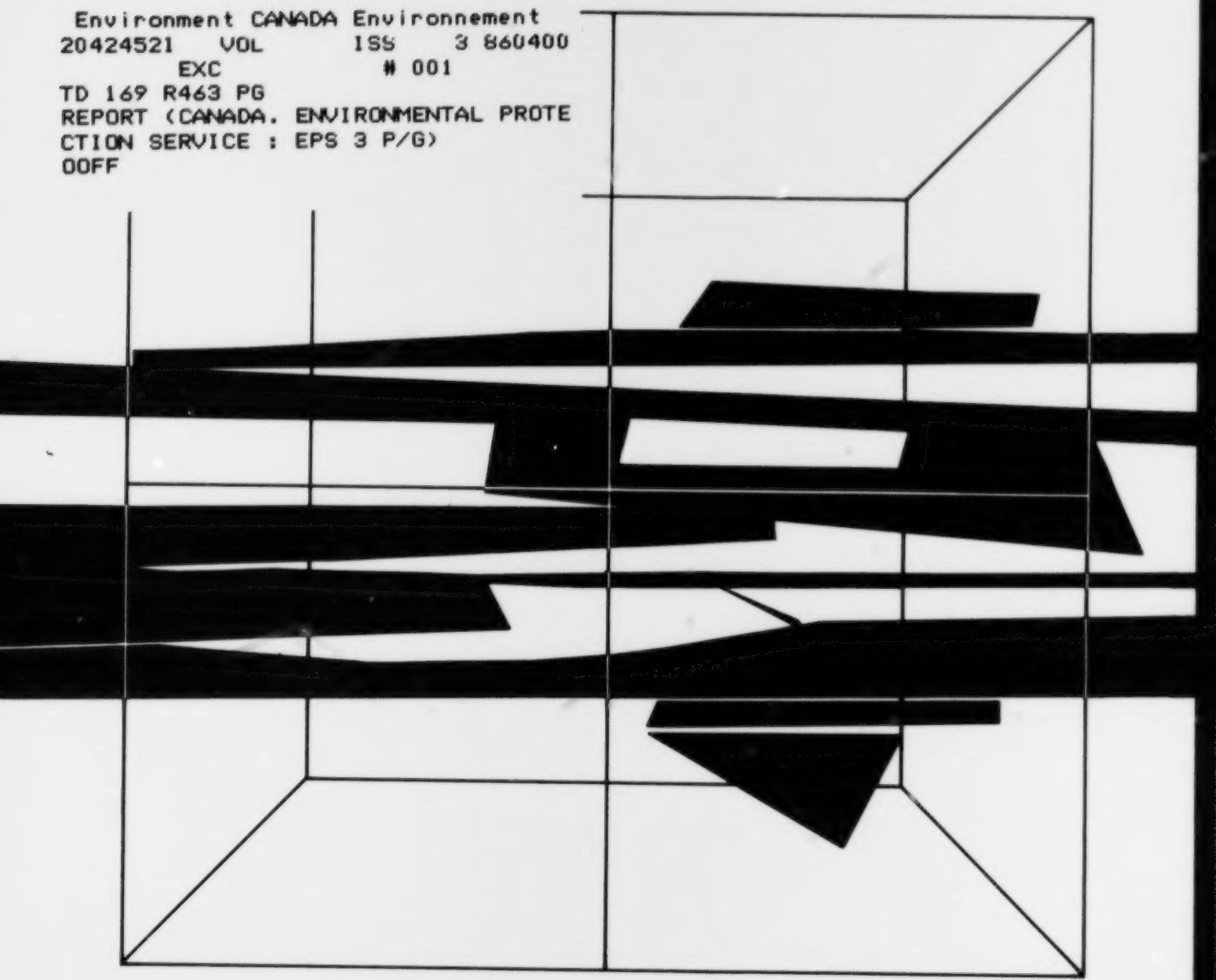

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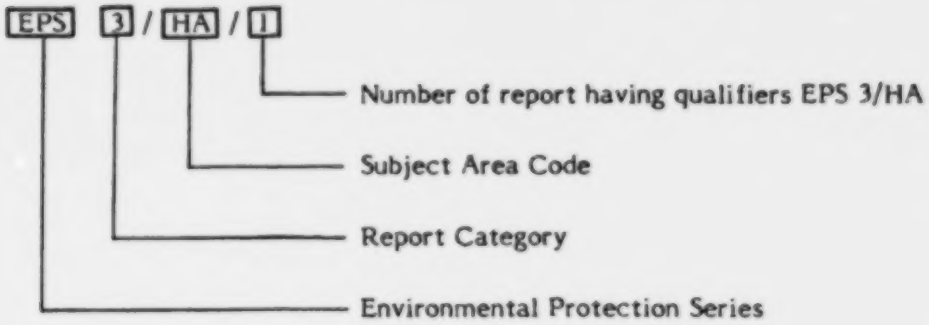

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CHARACTERIZATION OF PCF POWER STATION COMBUSTION WASTES USING TOTAL ORGANIC HALIDE ANALYSIS AND TOXICITY SCREENING TESTS

by

Dearborn Environmental Consulting Services

for the

Industrial Programs Branch
Environmental Protection Programs Directorate
Environment Canada

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ABSTRACT

The characterization of coal combustion wastes using a battery of analytical protocols was examined. Aqueous and, to a limited extent, solvent extracts from representative Canadian coal residues at pulverized coal fired power generating stations and from an atmospheric fluidized bed combustion unit were characterized. Emphasis was placed on the characterization of aqueous extracts because these would likely be more representative of the leachates that are released from the wastes.

The program consisted of screening for organohalides and short-term toxicity testing. The organic components were quantified using total organic carbon (TOC), total organic halide (TOX), and purgeable organic halide (POX) analyses. The toxicity screening procedures were the Microtox™, an experimental adenosine triphosphate (ATP)/Polyseed™ and a rapid algal method. In terms of the inorganic constituents, the aqueous extracts were similar in character to the results reported by other researchers. Toxicity could not be correlated to the presence of organohalides primarily because of their very low or nondetectable concentrations in the aqueous extracts, but there was a high correlation between toxicity as determined by the Microtox and ATP/Polyseed methods. Increased toxicity was highly correlated to increased pH, and lower toxicity was associated with higher concentrations of certain elements.

The characterization of coal combustion residues using a screening procedure involving organic halide analyses, a battery of short-term toxicity test and inorganic chemical characterization appears to be promising, although several limitations and areas requiring refinement are identified.

RÉSUMÉ

On a examiné une série de méthodes utilisables pour la caractérisation des résidus de la combustion du charbon. Des extraits aqueux et, à un degré moindre, des extraits dans un solvant, des résidus produits par des charbons canadiens représentatifs dans des centrales à charbon pulvérisé et à combustion atmosphérique sur lit fluidisé ont été utilisés à cette fin. L'accent a été mis sur les extraits aqueux, lesquels devraient être plus représentatifs des eaux de lessivage de ces résidus.

Le programme a consisté à mesurer les organohalogénures ainsi que la toxicité à court terme. Les teneurs en carbone organique totale, en halogénures organiques totaux et en halogénures organiques solubilisables ont été mesurées. Trois méthodes de dépistage de la toxicité ont été employées: la méthode Microtox™, une méthode expérimentale à l'adénosine triphosphate (ATP)/Polyseed™ et une méthode rapide sur algues. En ce qui concerne les constituants inorganiques, les caractéristiques des extraits aqueux étaient semblables à celles qu'ont trouvées d'autres chercheurs. On n'a pas pu établir de corrélation entre la toxicité et la présence d'organohalogénures en raison surtout de leurs concentrations très faibles, ou non décelables, dans les extraits aqueux, mais une corrélation élevée a été mise en évidence par les méthodes Microtox et ATP/Polyseed pour la toxicité. Une corrélation élevée a été observée entre l'augmentation de la toxicité et l'augmentation du pH; de plus, une toxicité plus faible était associée à des concentrations plus fortes de certains éléments.

La caractérisation des résidus de la combustion du charbon par une méthode de dépistage comportant des analyses des organohalogénures, une série de tests de toxicité à court terme et la caractérisation des constituants chimiques inorganiques semble une technique prometteuse, quoique plusieurs limites et points à améliorer ont été reconnus.

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1 INTRODUCTION

Residues from the combustion of coal have recently become the subject of detailed chemical characterizations which have included both inorganic and organic parameters. These studies indicate that ashes may contain low concentrations of specific organic compounds. Although these findings are valuable in quantifying a limited number of specific organic compounds, the procedures used can be extremely complex and costly. They may involve solvent extraction of the combustion residue and sophisticated instrumentation operated by skilled chemists. This emphasis on specific chemical analyses has, at times, diverged from the original notion of characterization studies as a means to categorize residues by their effect on the environment in order to better manage and dispose of them in an environmentally safe manner.

While some life forms can exist in a suspended state in a non-aqueous environment, all organisms require water to function actively. From this perspective, the characterization of combustion residues must also include the water soluble compounds leached from the wastes. Current water quality criteria are based on total recoverable rather than dissolved concentrations of toxicants. Historically, only total concentrations were reported in the published results of aquatic bioassay testing, even though it was generally known and accepted that the dissolved fraction is the most bioavailable and toxic, and that certain dissolved species are much more toxic than others. With the current emphasis on the chemical characterization of solid residues for organics, attention has drifted away from the characterization of ash-contacted waters and the measurement of the toxicity of such wastewaters to biological systems or living organisms.

Most standards for environmental protection are based on a series of assumptions. For example, the response of a test species may be extrapolated to a standard for protection of the multiplicity of life in an entire ecosystem against that toxicant. If the assumptions are incomplete or incorrect, such as the omission of a high risk member of the food chain, the standards based on these assumptions will be inappropriate for protection. A standard for an environmental toxicant that is based on only one toxicity screening method may be overprotective or underprotective for ecosystems with differing biotic and physiochemical properties. A battery of protocols would more thoroughly assess the effects of a toxicant at several points in the ecosystem. Logistical considerations would dictate that such a battery of tests should be widely practicable, available and reasonably quick.

In a similar way, organic compound characterizations must include a wide range of chemical structures, such as polar and nonpolar, volatile or non-volatile, and cyclic or straight-chain molecular structure. Logistics would also dictate that screening procedures or surrogate parameters could be employed to identify the aggregate toxicity of organics in a wastewater and could be used to identify the need for more specific analyses. From a different perspective, one screening procedure could be used to demonstrate the absence of organic compound contamination, reducing the need to resort to a wide variety of specific methods which would otherwise be needed to eliminate all possibilities.

From an ecological and logistical viewpoint, the optimal means to characterize coal combustion residues would consist of surrogate parameters to quantify all water leachable species and a battery of toxicity screening procedures to quantify their effect on several links in the ecosystem. This report describes a study to investigate the application of organic halide analysis as a surrogate parameter in combination with three short-term acute toxicity procedures.

The overall objective of this study was to examine characterization of coal combustion wastes using a representative battery of protocols. The more detailed objectives were to:

- conduct a critical literature review for the application of total organic halide analysis as a predictable screening method for organics of concern in coal combustion wastes;
- review the literature on short-term microbiological toxicity screening procedures emphasizing their practicability for characterization of wastes;
- characterize appropriate samples of coal combustion residues using total organic halide analysis, microbiological toxicity screening and conventional chemical characterization methods;
- determine what relationships, if any, exist between the results of the chemical and biological characterizations and between the characterizations and coal combustion residues; and
- present the results of the study and their interpretation, the practicability and utility of the methods used, as well as any conclusions and recommendations for further study.

2 LITERATURE REVIEW

This section is a critical review of the technical literature describing analytical methods for organic halide analysis. Both specific analyses and the use of surrogate parameters are described. Also, a critical review of short-term toxicity screening methods is presented with an emphasis on comparing the available techniques and assessing their practicability.

2.1 Analytical Methods for Organic Halide Analysis

Two different approaches are possible for the analysis of organic halides. These are specific analyses for trace quantities of organics by specialized techniques, or the use of surrogate, collective or group parameters. The first approach may use gas chromatography and mass spectrometry (GC/MS) which provide very detailed information on organic compounds that are identified and quantified. The determination procedure usually begins with an enrichment step, sometimes in conjunction with chemical derivitization, and requires sophisticated GC/MS equipment with a data processing system operated by skilled personnel.

The alternative is the use of surrogate parameters such as total organic carbon (TOC) or total organic halogen (TOX). These provide more general information about the nature of the sample. Such parameters can be determined in a short time with relatively simple equipment; however, their disadvantage is the lack of information concerning the multitude of specific organic compounds that may be present.

Overfield and Winefordner (1) described a sensitive detector for the selective detection of nanogram quantities of halogenated compounds. The detector consisted of a stainless steel burner into which the sample and air were introduced, and a concentric quartz jacket into which H_2 was introduced. A primary flame was supported on the stainless steel burner head and a secondary flame at the tip of the quartz jacket. The sample entering the primary flame is decomposed and the decomposition products pass over a screen containing indium. Any sample containing chlorine, bromine or iodine produces InCl, InBr or InI, respectively. The emission from the secondary flame is monitored spectrophotometrically. The response was found to be linear for six decades above the detection limit for chlorinated compounds. The researchers concluded that the indium halide detector should be useful for selective detections of nanogram quantities of halogenated organic compounds.

Gutsche, Herrmann and Ruediger (2) utilized the InCl emission in a flame as a sensitive, selective flame photometric method to determine chlorinated pesticides in butter.

Nulton, Haile and Redford (3) described a rapid, sensitive procedure utilizing a short GC column and a Hall electrolytic conductivity detector for measuring total organic halogen. The procedure was used to screen a large number of samples for total organic halogen for subsequent GC/MS analysis. Aqueous samples were extracted serially with three portions of cyclohexene, dried with sodium sulphate and concentrated to 2 mL by the Kuderna-Danish (K-D) method. The extract was then dissolved in 200 µL of benzene. Solid samples were subjected to Soxhlet extraction with benzene for 16 hours. The resulting extracts were handled in the same way as aqueous samples. The total organic halogen concentration was determined by short column GC with a Hall detector. Quantification was accomplished by comparing the area response of the sample chromatogram to that of the external standard, monochlorobiphenyl.

The table below summarizes the results of the study.

Sample Type	Source*	Number of Determinations	Range**	Mean**	Standard Deviation
Fly ash	1	51	0.3-10.6	4.0	2.4
	2	53	< 0.1-370	104	80
Bottom ash	1	51	0.2-362	63	85
	2	50	< 0.1-85	9.3	22
Coal	1	6	4.7	4.7	1.2
Bottom ash quench water	1	52	90-1180	705	230

* Source 1 - co-fired (coal and refuse derived fuel) power plant.
Source 2 - resource recovery municipal refuse incinerator.

** Results expressed in ng organohalides per gram for solid samples and ng organohalides per litre for liquid samples.

The total organic halogen concentration was quantified in nearly all samples, but specific chlorinated organics were detected by high resolution GC/MS (HRGC/MS) only in a small number of ash samples (4). In most cases the total organic halogen levels determined by the GC/Hall method compare well with the concentrations calculated for the samples in which significant levels of specific chlorinated compounds were identified by HRGC/MS. For example for fly ash from source 2, the short column/Hall method yielded 98 ng/g of total organic halogens while the HRGC/MS method gave 93 ng/g.

In terms of sensitive and specific analytical methods for the quantification of total organic halogens, the most widely favoured is high resolution gas chromatography coupled with mass spectrometry. In this method, a gas chromatograph which separates compounds is coupled with a mass spectrograph which is used for compound identification to provide quantitative data to very low concentrations of specific organic compounds. Detailed descriptions of this analytical method, equipment and laboratory procedures are found in a large number of textbooks. This procedure may be limited since not all halogenated organics are amenable to identification by GC. During the assessment of the quality of drinking waters where the interactions of chlorine and organic matter lead to the formation of many organic halides, it was found that a wide variety of organic halides were not determined by GC techniques. These included many polar or volatile compounds (5). Also, reference standards may not be available for all organic compounds of interest. Of greater limitation in a practical sense are: the high cost of individual analyses, the rigorous laboratory procedures involved, and the skilled training of the equipment operators.

Other analytical techniques for the specific quantification of organic halogens have been suggested. Flash vacuum-evaporation is another method under study (6). It involves first a desalting step via steam generation and recondensation followed by the catalytic conversion of organohalides to Cl_2. The Cl_2 is then measured by a fluorometric derivitization analysis using syringaldazine (7).

Neutron activation (NA) followed by gamma ray spectroscopy has been used for the analysis of organohalides. This technique was first reported in Sweden (8). The organic halides can be isolated by either solvent extraction or carbon adsorption. Although NA can differentiate between the halogens that comprise TOX, this does not appear to be a routine analytical tool (7).

Finally, the ion chromatograph offers another possibility for the detection and measurement of individual halides such as would be recovered in a pyrohydrolyzate and reported as organic halides (9). These methods do not appear to have been widely employed, particularly in application to combustion residues.

Because of the extremely large number of organic halides potentially present and the broad spectrum of environmental media in which organic halides occur, rapid and cost-effective screening have been sought. Total organic carbon (TOC) is a measure of the organic carbon in wastes and wastewaters. Some of these carbon compounds can be oxidized further by biological or chemical processes and the biological oxygen demand

(BOD) and chemical oxygen demand (COD) can be determined. Unlike BOD and COD, the TOC is independent of the oxidation state of the organic matter and is not a measure of other organically bound elements such as nitrogen or hydrogen that may contribute to COD or BOD (10).

In the TOC analysis, organic carbon is oxidized to carbon dioxide (CO_2) by heat and oxygen, ultraviolet irradiation, chemical oxidants or various combinations of these. The CO_2 may be measured directly by a nondispersive infrared analyzer or it may be reduced to methane and measured by a flame ionization detector in a gas chromatograph. The CO_2 may be titrated chemically (10).

Harrison, Bishop and Mallon (11) determined the TOC in solid combustion residues from a low-sulphur sub-bituminous coal fired generating station in New Mexico. The TOC concentration was determined using the persulphate oxidation method (12). Aqueous samples were filtered through precombusted glass fibre filters while predried sediment samples were directly analyzed, in triplicate, using a carbon analyzer.

The organic carbon concentrations, expressed in parts per million (ppm), are shown below. Values are means of three analyses with a fractional standard deviation less than or equal to 0.03.

Sample	DOC[a]	POC[b]	DOC + POC	TOC[c]
Scrubber water (filtered in field)	2.8	-	-	-
Scrubber water (filtered in lab)	2.2	-	-	-
Ash pond	3.3	2.4	5.7	4.4
Electrostatic precipitator fly ash	-	-	-	170
Scrubber fly ash	-	-	-	260
Ash pond solids	-	-	-	330

a Dissolved organic carbon (DOC) in the organic carbon in the filtrate.
b Particulate organic carbon (POC) in the organic carbon captured on the filter.
c Total organic carbon should be equal to DOC + POC.

An increasingly used surrogate procedure to quantify organic solids is the pyrolysis/microcoulometric technique which involves:

1) adsorption of the organic halides from water onto activated carbon;
2) removal of the organic halide from the carbon by rinsing with a concentrated potassium nitrate solution;

3) combustion of the carbon at 800°C in an oxygen atmosphere with collection of the hydrogen halide (HX) combustion products in an acetic acid solution; and,

4) microcoulometric titration of the captured HX using silver ion.

This analytical approach is designed to detect virtually all organic halides, since carbon adsorption is a generally effective removal technique, and the result obtained using this technique is commonly referred to as total organic halide (TOX), although carbon adsorbable organic halide (CAOX) is a more accurate definition (5).

A related method for determining purgeable organic halide (POX) involves purging the organic halides from the sample directly into the pyrolysis furnace with a carbon dioxide stream. Instrumentation for conducting both TOX and POX techniques has been developed.

The American Society for Testing and Materials describes a proposed test method for organic halides in water by carbon adsorption microcoulometric detection. This method is intended to quantify organic halides in the concentration range of 5 to 1000 µg/L. It was noted that organic halides typically do not occur in natural waters at concentrations greater than 5 µg/L. The greatest source of variability in this method is probably the granular activated carbon which should ideally have very low apparent halide background, readily release any absorbed inorganic halides upon nitrate washing, and reliably adsorb organic halides even in the presence of a large excess of other organics (13). This method was originally developed by Kuhn and Sontheimer (14,15) as a means to measure halogenated organic compound removal by granular activated carbon filters at water treatment plants along the Rhine River and was extensively evaluated and refined by Dressman and co-workers (9,16). Their principal contribution was the use of microcoulometry to measure the chloride ion since selective ion probes previously used were shown to have a positive bias. The type of detector has significantly increased the sensitivity, reducing the necessary sample size.

Riggin et al. (5) studied this analytical technique in application to total organic halide quantification in solid wastes, wastewaters and solvent extracts of these. These wastes included effluent from an aniline manufacturing facility, effluents from chlorinated hydrocarbon manufacturing facilities, and organics leachate from a ferrous-chloride spent-catalyst waste.

The researchers concluded that the method was well suited for wastewater analysis. They also investigated the use of solvent extractable total organic halogen

(EOX) methods for use in cases where only extractable haloginated chemicals were of concern. This approach would not determine polar organic halides.

The method, applied to water samples, involved extraction with three aliquots of cyclohexane and concentration of the extract by the Kuderna-Danish (K-D) method. For solid residue, extraction was completed with a polar organic solvent followed by direct analysis of the solvent extract. The researchers concluded that the EOX/solids method appeared to work well and they reported recoveries of 47 percent to 105 percent depending on the specific organohalide.

An alternative procedure for determining TOX has been proposed (17). Organic constituents are concentrated onto Amberlite XAD resin rather than activated carbon followed by solvent desorption from the resin, controlled combustion of a portion of the solvent sample and detection by microcoulometry. This approach was used to determine the halogenated organic constituent contents of water supplies; however, the resin demonstrated unfavourable adsorption characteristics for some compounds.

Sederka and Lechner (18) described the use of a porous polymer, Tenax GC, to adsorb organohalides from natural waters prior to thermal desorption. This polymer was used because it was stable up to 400°C, in comparison to Amberlite XAD which was stable to 275°C (19). This polymer was investigated as an alternative to activated carbon which must be washed with nitrate solution, and which contains some halogens that are not removable (20). The efficiency of adsorption was established by analyzing synthetic samples of waters containing known amounts of organohalides. Recoveries of ten model compounds averaged 80 percent at 1.0 ppb (parts per billion) levels. It was found that sulphur and nitrogen interferences were eliminated and the sensitivity, recovery and precision compared well with other methods. The method was found to be suitable for routine analyses of natural water; however, the results of analyses should be expressed as Tenax adsorbable organohalides in natural water.

The carbon adsorption pyrolysis/microcoulometric technique has been used to determine the organic halogen content of several types of industrial wastewaters. Carlberg and Kringstad (21) investigated this and two other methods in application to effluents from bleacheries in the pulp and paper industry. This technique was felt to be appropriate because these wastewaters contain a broad spectrum of chlorinated organic substances, including aliphatic and aromatic hydrocarbons, aldehydes, ketones, acids, phenols, thiophenes and higher molecular weight lignin degradation products. Compared to CAOX, extraction with butylacetate followed by neutron activation analysis could account for only 18 and 38 percent of the organic chlorides in the two samples studied

(21). It was believed that the extraction procedure determined only those chlorinated compounds which were extractable, that is, mainly low molecular weight compounds. The detection limit of the method was about 10 µg/L of organic halogens.

Several researchers have applied total organic halogen determinations to characterize reclaimed and chlorinated waters. Jekel and Roberts (20) used this method to characterize reclaimed waters consisting of secondary effluent from activated sludge treatment followed by high lime treatment, ammonia stripping, recarbonation, chlorination, dual media filtration and granular activated carbon adsorption. Reinhard (22) used this technique to monitor the performance of ultrafiltration of advanced treated wastewater and in groundwater. Chow and Roberts (23) used the procedure to quantitatively measure the halogenating characteristics of chlorine dioxide and chlorine as wastewater disinfectants.

Quinn and Snoeyink (24) monitored the removal of total organic halogens from wastewater by granular activated carbon absorbers using carbon adsorption/pyrolysis/microcoulometric methods. Finally, McCarty and Aieta (25) concluded that total organic halogen determination by this method appeared to be a good surrogate parameter for monitoring the effectiveness of treatment systems because it can be measured readily, reliably and rapidly.

The methods for preservation of total organic halides formed from chlorinated humic acid were investigated by Tsai and Tuovinen (26). Acid addition to pH less than 2 was found to be more effective than treatment with azide or molybdate bactericides. These researchers noted that membrane filtration under vacuum accelerated the volatilization of purgeable organic halogens in the samples. This type of filtration also slightly reduced the concentration of total organic halogens in the sample. Changes in TOX concentrations in preserved samples with time were also studied. An eight-percent reduction in total organic halogens was found after a 28-day holding period in acidified environment.

A limited number of studies have been conducted to characterize the organics present in coal combustion residues. These have employed both specific and surrogate analytical methods.

Hanson, Carpenter and Newton (27) characterized organics in vapour phase and those extracted from the bag ash of an experimental fluidized bed combustor (FBC). The concentrations of vapour phase hydrocarbons from the combustion of Texas lignite in the FBC, shown below, were captured on Tenax and subsequently were subjected to Soxhlet extraction with pentane.

Sampling Location	Hydrocarbons with more than six carbon atoms (mg/m^3)
Before primary cyclone	3.3 - 33
Before bag filter	1.0 - 3.0
After bag filter	0.5 - 3.0

Using glass capillary gas chromatography/mass spectrometry, hydrocarbons collected before the primary cyclone were found to have molecular weights ranging from 128 to 248 and were tentatively identified as napthalene, methylnapthalene isomers, phenanthrene or anthracene, 2,6-Bis (1,1-Dimethylethyl) -4- methylphenol and 2,5-Bis (1,1-Dimethylpropyl) -cyclohexadiene- 1,4-dione. Compounds tentatively identified after the bag filter had molecular weights ranging from 118 to 184 and included 3 and 4 carbon benzene compounds, napthalene, methylnaphthalene isomers, dibenzofuran, phenanthrene or anthracene and dibenzothiophene.

Extractable hydrocarbons in three bag ashes using methylene chloride with ultrasonic extractions are show below.

Coal Burned	Extractable Hydrocarbons ($\mu g/g$)
Texas lignite	17 - 54
Western Kentucky sub-bituminous	340
Montana Rosebud sub-bituminous	1.9 - 11

Using the same extraction procedure, hydrocarbons from sized particles of fluidized bed combustion wastes from western Kentucky sub-bituminous coal were determined. No significant relationship between extractable hydrocarbon concentration and particle size was found.

Harrison, Bishop and Mallon (11) recovered organic compounds from solid and liquid wastes from the Four Corners generating station in New Mexico. The station burns low-sulphur sub-bituminous coal. Organic constituents in water and solid samples were extracted with three portions of methylene chloride and were characterized by gas chromatography with mass spectrometry. Liquid wastes contained low molecular weight alkanes, alcohols, ketones and phthalates. Organic constituents in extracts of the solid

wastes included many aliphatic and aromatic compounds. Aliphatic fractions contained C_{15} to C_{30} paraffins while aromatic fractions contained one, two, three and four rings. No compound contained more than four rings. The researchers concluded that although a large number of compounds were identified, concentrations were generally low. Some of the compounds could also have resulted from the extraction procedure. In addition, some of the compounds could not be identified by GC/MS because known spectra are not available for them.

Very limited data exist on the characterization of combustion residues from Canadian coals for organic halogens. Coburn et al. (28) determined the total extractable organic halogen content (TEOX) of fly ashes from three Canadian coal fired generating stations. Prior to analysis, the ash samples were acidified with HCl and shaken for two hours. Following settling, the supernatant was filtered and the filtrate was rinsed with organic free water and filtered as before. The xHCl and water rinses were then extracted in a separatory funnel with toluene. The toluene extract of the acids and rinses was used to extract the ash for 24 hours with a cycle time of 15 minutes. Following this, the toluene solution was removed and reduced in volume to 2-3 mL on a rotary evaporator with solvent exchange into isooctane. The extract was quantitatively transferred to a calibrated centrifuge tube and made up to 5 mL. TEOX analysis was performed by the injection of 1 to 5 µL into a Dohrmann MCTS-20 microcoulometer. The table below shows the calculated concentration of TEOX in the ash samples based on extract concentration and the ash weight represented by that portion of the extract.

Site	Number of Determinations	Coal Type	TEOX (µg Cl^-/g) Mean	Standard Deviation	Coeff. of Variation
1	1	lignite	5.25	-	-
2	14	low sulphur sub-bituminous	0.9*	1.56*	1.73
3	8	high sulphur bituminous	4.74	4.89	1.03

* excludes one value of 234 µg Cl^-/g which was considered to be an outlier.

For purposes of comparison, the TEOX of urban dust samples (National Bureau of Standards, standard material 1649) were analyzed in duplicate. The TEOX was found to be 1383 ± 105 µg Cl^-/g.

In summary, both specific and surrogate methods have been used to characterize wastewaters and solid residues for organic halogens. Specific methods have primarily used GC/MS techniques which have quantified relatively low concentrations in coal residues and related wastewaters. In general these procedures have involved a solvent extraction and concentration step prior to identification and quantification. Not all compounds can be identified since spectra for many compounds are not known. It has been suggested that the solvent extraction may in part result in contamination of the samples prior to analysis, resulting in procedural artifacts. Surrogate parameters include TOC and total organic halide analysis. These have been developed as a result of the time, high cost, and complex equipment requirements of the more specific techniques. Solvent extraction has been used prior to quantification; however, several researchers have pointed out that not all organic compounds will be extracted by this method, particularly low molecular weight polar compounds.

The most promising surrogate method for organic halide quantification appears to be carbon adsorption/pyrolysis/microcoulometery. It has been used successfully to monitor chlorination systems and to characterize organics in industrial and domestic wastewaters. In one case, this procedure was used to quantify organic halogens extracted with solvents from coal fly ash. Analysis of coal combustion wastes without solvent extraction, or of aqueous extracts, does not appear to have been, as yet, reported. This is probably due to the assumption that if organic compounds cannot be removed by vigorous extraction with an organic solvent such as methyl chloride, it is unlikely that they would be removed by a nonorganic solvent, i.e., water (11). The validity of this has yet to be proven.

Table 1 summarizes TOC and organic halide concentrations found in coal, combustion wastes and related wastewaters. The interpretation of this data is difficult because of the wide spectrum of sample types and sources, and the various analytical techniques and methods of reporting results.

In only one study (11) is it possible to compare the TOC of solid residues with that of the related wastewater. For the one sub-bituminous coal ash, the TOC of the fly ash was about 75 times greater than the ash pond water. For the scrubber fly ash, the TOC in the solid was about 100 times greater than in the related wastewater. Although the details of the system design such as ash/water contact time and degree of mixing were not provided, it would appear that only a small percentage of the TOC is mobilized from the solids to the water.

TABLE 1 TOC AND ORGANIC HALIDE CONCENTRATIONS IN COAL, COMBUSTION WASTES AND RELATED WASTEWATERS

Sample Type	Sample Description	Sample Source	Number of Determinations	Analytical Technique	Result	Reference
Solid	Fly ash	cofired (coal and refuse) power plant	51	Solvent extraction/ short column GC/MS	0.3 - 10.6 ng/g	3
		municipal refuse incinerator	53	Solvent extraction/ short clumn GC/MS	<0.1 - 370 ng/g	3
		ESP ash - subbituminous coal	unknown	TOC*	170 µg/g	11
		AFBC bag ash Texas lignite	several	Solvent extraction/GCMS	17 - 54 µg/g	27
		AFBC bag ash Kentucky subbituminous	several	Solvent extraction/GCMS	340 µg/g	27
		AFBC bag ash Montana subbituminous	several	Solvent extraction/GCMS	1.9 - 11 µg/g	27
		ESP ash - lignite	1	Solvent extraction/TEOX	5.25 µg Cl/g	28
		ESP ash - subbituminous	14	Solvent extraction/TEOX	0.9 µg Cl/g	28
		ESP ash - bituminous	8	Solvent extraction/TEOX	4.74 µg Cl/g	28
	Bottom ash	Cofired (coal and refuse) power plant	51	Solvent extraction/ short column GC/MS	0.2 - 362 ng/g	3
		Municipal refuse incinerator	50	Solvent extraction/ short column GC/MS	<0.1 - 85 ng/g	3
	Coal	Coal type unknown	6	Solvent extraction/ short column GC/MS	4 - 7 ng/g	3
	Scrubber fly ash	subbituminous coal fired power plant	unknown	TOC	260 µg/g	11
	Ash pond solids	subbituminous coal fired power plant	unknown	TOC	330 µg/g	11
Waste-water	Bottom ash quench water	Cofired (coal and refuse) power plant	52	Solvent extraction/ short column GC/MS	90 - 1180 ng/L	3
	Scrubber water	subbituminous coal fired power plant	unknown	DOC	2.8 mg/L	11
	Ash pond water	subbituminous coal fired power plant	unknown	TOC	4.4 mg/L	11

*TOC = total organic carbon, DOC = dissolved organic carbon.

Where specific chemical analyses cannot measure, or cannot easily measure, halogen-containing chemicals, TOX can be useful as an indicator. In fact, TOX has been incorporated into U.S. federal regulations for monitoring groundwaters near hazardous waste sites (29). Although the determination of TOX can, in some cases, be limited, Steven et al. (7) identified its unique advantage. It can demonstrate the absence of organohalides contamination without the need to resort to a variety of specific methods to eliminate all the possibilities. Substantial cost savings result when one analytical method can be used.

2.2 Short-term Toxicity Screening Using Microbial Systems

While characterization techniques based on physical/chemical analyses are very valuable, they have shortcomings. Physical/chemical methods can only provide information about the presence of known toxicants under known physical conditions. Therefore, a sample could be deemed non-hazardous if chemicals not known to be toxic were present or if the physical/chemical testing did not include them. In addition, the presence of certain chemical compounds may interfere with the analytical detection methods. Physical/chemical testing does not measure synergistic effects where two or more chemicals act together to produce an increased toxic effect. Moreover, information concerning the mechanism of toxicity is not provided.

Supplementing physical/chemical analyses with bioassays could overcome these limitations but, until recently, bioassays have not been practical on a routine basis. Standard bioassays can be very expensive and time-consuming, require special skills, and often can be difficult to quantitate. These difficulties have prompted the development of toxicity screening tests which use microorganisms. The basic principle in all these tests is that there are fundamental life-sustaining biochemical processes common to all organisms and that the toxic chemicals interfere with these processes.

Research in the use of microorganisms for toxicity testing has demonstrated many advantages. These tests can be rapid, with effects often observed within minutes. They can be relatively inexpensive, and can require less labour than other bioassay techniques. Because microorganisms are small and replicate quickly, millions of these organisms can be used in each test to obtain superior statistical accuracy. Because their growth can be suspended and they can be reactivated quickly, a more standardized test with a standardized biological reagent can be achieved. Since microorganisms grow in a relatively wide range of environments, increased flexibility in the test conditions can be arranged. In addition, the small size of microorganisms readily permits miniaturization of

test procedures, using small sample sizes. The sensitivity of a test can also be increased significantly by sample concentration.

The use of microbial toxicity tests is gaining momentum. In May 1983, the First International Symposium on Toxicity Testing using Bacteria was held at the National Water Research Institute in Burlington, Ontario. Representatives from a dozen countries participated and arrangements for future symposia and a scientific periodical on this subject have been completed. A central theme at the symposium was the requirement for determining a set of tests so that the applicability of one type of test would complement the limitations of the others. There was considerable optimism that such a battery of tests could provide a good screening method so that the need for more costly and time consuming tests could be greatly reduced.

The following sections present a critical overview of this rapidly developing technology. The highlights of this overview have been summarized in Table 2.

2.2.1 The Microtox™ System. One of the most well developed toxicity bioassays using bacteria is the Beckman Microtox system. This system standard uses freeze-dried cultures of a strain of Photobacterium phosphoreum, a luminescent bacterium. Under normal conditions, the organisms continuously produce light which can be measured with a photometer. The bioluminescence of this organism can be considered a branch of its electron transport system as it is coupled via reduced flavin mononucleotide $FMNH_2$ (30). $FMNH_2$ is one of the co-substrates of the enzyme luciferase which catalyzes the luminescent reaction in photobacteria. When low levels of a toxicant interfere with the organism's metabolism, its light output decreases. Since the light reduction is proportional to the amount of toxicant present, the relative toxicity of a sample can be calculated from photometer readings.

The Microtox assay is a relatively simple test requiring no special skills. The test organism is supplied in freeze-dried form and only requires 15 minutes incubation after hydration before use. Labour for cultivation and maintenance of a test bioassay is eliminated as are sources of error from this aspect of bioassay testing (assuming good quality control by Beckman during manufacturing and shipping). Essentially all reagents and materials are available from Beckman, and the test procedure in Beckman's manual is highly formalized. Conditions for a standardized test with a standard organism from laboratory to laboratory are maximized.

A moderate capital investment is required for the purchase of a Beckman Toxicity Analyzer (or for an equivalent system) and the lyophilized culture vials.

TABLE 2 COMMON SHORT-TERM TOXICITY SCREENING TESTS USING MICROORGANISMS [a]

Bioassay Method	Capital and Operational Costs	Labour	Time Required	Reproducibility[c]	Cell Mass Requirements	Comments
1. Microtox™	moderate	No specialized skills required. Requires good micropipetting technique.	<60 min	good	relatively small amounts	Superior in simplicity and speed. Often superior to other microbial tests in sensitivity to toxicants but significant exceptions occur. Generally less sensitive than fish bioassays. Only microbial bioassay system in which the organism is a commercial product. Available from only one supplier.
2. ATP: as a viability test	moderate	No specialized skills required for analysis[b]. Requires good micropipetting technique.	<5 min per sample when testing endogenous populations	good	relatively small amounts	Capable of measuring smaller amounts of biomass than any other rapid assay. Can measure any species of microorganism. Successfully applied in measuring of effects of adverse environments to endogenous populations. Adequate sensitivity to toxicants has not yet been demonstrated by measuring loss of viability of surrogate microorganisms with the ATP assay. Instrumentation is available from several commercial sources. The instrumentation can be used to perform other types of toxicity tests including Microtox™.
3. ATP: as a growth inhibition test	moderate	No specialized skills required[b] for analyses. Requires good micropipetting technique.	<60 min following 1 to 2 h incubation (longer incubations are required for testing slow growing organisms)	good	relatively small amounts	Provides a mechanism to overcome lack of sensitivity of surrogate organisms observed with ATP viability testing but retains all the advantages and is simpler. Automation of the analyses could readily be achieved with commercially available instrumentation.

TABLE 2 COMMON SHORT-TERM TOXICITY SCREENING TESTS USING MICROORGANISMS [a] (Cont'd)

Bioassay Method	Capital and Operational Costs	Labour	Time Required	Reproducibility[c]	Cell Mass Requirements	Comments
4. Inhibition of growth: turbidmetric analyses	low	No specialized skills required for analyses[b].	<60 minutes after several hours to overnight incubation (longer incubations are required to test slow growing organisms)	good	large amounts required	This type of test is appealing from the perspective of cost and simplicity, but the requirement for high biomass may often lead to insensitive measurement of toxicity.
5. Inhibition of growth: plate count testing	low	Requires aseptic technique capability.	One to several days	good	small amounts required	The long incubation periods of a protocol using plate counts reduces its attractiveness as a short-term assay.
6. Respiration assays by dissolved oxygen analyses[b]	low	No specialized skills required for analyses[b].	60-120 minutes after appropriate exposure to toxicant (minutes to hours)	good	large amounts required	Dissolved oxygen meter is a common instrument in wastewater laboratories. The high biomass requirement may often lead to insensitive measurement of toxicity.
7. Algal assays: GENERAL	see below	see below	see below	see below	see below	Algal assays are important to indicate in a primary battery of toxicity screening tests because they are representative of primary producers in the food chain. Other microbial assays cannot detect toxicants which primarily affect photosynthesis. Slow growth of algae causes the need for relatively long incubation periods and for greater care in providing uniform testing conclusions.
8. Algal assays: microscopic enumeration	low	No specialized skills required for analyses[b]. Tedious and labour intensive.	60 to 120 min following several days of incubation	Often prone to errors. Not applicable to filamentous species.	moderate amounts for determination of end point	Widely used because of low expense. Morphological changes caused by toxicants also detected.

TABLE 2 COMMON SHORT-TERM TOXICITY SCREENING TESTS USING MICROORGANISMS [a] (Cont'd)

Bioassay Method	Capital and Operational Costs	Labour	Time Required	Reproducibility[c]	Cell Mass Requirements	Comments
9. Algal assays: electron counting cell	high capital, low operational	No specialized skills required for analyses[b].	<60 min follows several days of incubation	Good but not applicable to filamentous species.	relatively small amounts	Does not discriminate between living cells, dead cells and debris of similar size to the cells.
10. Algal assays: dry weight measurement	low	No specialized skills required for analyses[b]. Somewhat labour intensive.	60 to 120 min following several days of incubation	Often prone to errors.	large amounts for end point determination	Does not discriminate between living cells, dead cells and particulate matter.
11. Algal assays: oxygen evolution	low	No specialized skills required for analyses[b]. Somewhat labour intensive.	60 min following one to several hours incubation	good	large amounts required	High biomass requirement as in number 6.
12. Algal assays: radioactive techniques	high capital moderate operational	Handling of radioactive material requires appropriate personnel.	60-120 min following several hours to overnight incubation	good	Without using concentration procedure, moderately high amounts are required for rapid end point determination	Use of radioactive material and high cost of equipment discourages use in most small laboratories.
13. Algal assays: chlorophyll a	low to moderate capital, low operational	No specialized skills required for analyses[b].	60-120 min following incubation periods of several days	good	Moderate to small amounts required	Chlorophyll a measurement is specific for algal cells. Fluoremetric measurement of chlorophyll a is significantly more sensitive than spectrophotometric. Analysis significantly faster and easier if measurement from intact cells can be used. DCMu induced fluorescence methods may shorten incubation time significantly.

TABLE 2 COMMON SHORT-TERM TOXICITY SCREENING TESTS USING MICROORGANISMS [a] (Cont'd)

Bioassay Method	Capital and Operational Costs	Labour	Time Required	Reproducibility[c]	Cell Mass Requirements	Comments
14. Algal assays: ATP	moderate	No specialized skills required for analyses[b]. Requires good micropipetting technique.	<60 min following several hours to overnight incubation	good	Small amounts required	Significant quantities of other types of organisms interfere with analyses. Therefore, this assay is restricted to laboratory culture protocols. ATP appears to respond more rapidly to toxic stresses than chlorophyll.
15. Dehydrogenase assays: spectrophometric (e.g., reazarin, TTC, INT, etc)	low	No specialized skills required for analyses[b].		good	Large amounts required	Relatively attractive from a standpoint of cost and simplicity but the requirement for dense cultures can cause sensitivity problems in detection of toxicants.
16. Dehydrogenase assays: microscopic analysis	low to moderate capital, low operactional	Many applications make use of trained microscopist desirable. Tedious for large number of samples.	60-120 min following appropriate exposure (minutes to hours)	good	Many applications incorporate a concentration technique, reducing the amount of biomass required.	Appears practical in assessment of agents to prevent growth of bulking microorganisms in activated sludge. Applications in second-tier testing in microbial ecological tests also appear promising.

a This simplified evaluation represents the opinions of the authors and in some instances is open to qualification. Adequacy as primary screening tests requires supplementation.

b Unless a source of appropriate microorganisms is not available, aseptic cultivation skills may also be required.

c This assumes uniform test conditions for the microbial test organisms is provided.

However, in relation to fish bioassay costs and capital requirements, the expense of the assay is relatively low (31).

A strong advantage offered by the Microtox assay is its speed. The most commonly used test incubation periods for the Microtox bacteria with a toxicant are five and fifteen minutes. This makes it possible to complete the whole analysis within an hour although, depending on the degree of precision and the extent of pre-screening required, the time may be as long as two to three hours. This is still relatively short for a toxicity test. Less than an hour may be needed if only a crude pass-fail screening test is desired.

In addition, the Microtox assay is a relatively sensitive test for toxicants. For order of magnitude estimations of fish toxicity, the Microtox assay has performed well in most cases (31,32,33,34,35,36). Also, in a comparison with a Daphnia test study of industrial effluents, good agreement was found for most of the samples (37). In another study, conducted by the City of Chattanooga (38), it was concluded that the Microtox assay was a valuable tool for making judgements about the acceptability of industrial and special wastes for handling by a waste treatment plant.

Although a significant number and degree of exceptions were present, the Microtox assay has also been shown to be more sensitive than several other microbial tests, i.e., the Spirillum motility test, Pseudomonas fluorescens growth inhibition, inhibition of activated sludge (domestic and also synthetic) respiration, and inhibition of activated sludge dehydrogenase (TTC method) (39,40). Furthermore, in a study concerning biomaterials testing (32), the Microtox test appeared even more sensitive than the tissue culture cytotoxicity test. Because of its speed, relative simplicity and sensitivity, the rapid increase in the use of the Microtox system is not surprising.

While the commercialized nature of the Microtox assay provides the potential advantage of maximizing test standardization, the test is only available from one supplier, Beckman Instruments Ltd. While it is advantageous for the manufacturer to be sympathetic to its clients, the single-source supplier aspect of this test may cause some difficulties at times. In this project, delivery of the Microtox reagents took seven weeks and caused an inconvenient delay in the testing program.

Although the Microtox assay is quite sensitive for toxicity testing, it has some other shortcomings. As a surrogate parameter for fish toxicity, the test is often good as an order of magnitude indicator, but generally the fish bioassays are still more sensitive (31,34,35,36). Indorato et al. (41) reported examples where the Microtox test was over two orders of magnitude less sensitive than fish bioassays. Other investigators have also reported examples where bacterial assays have shown more than an order of magnitude

greater sensitivity than the Microtox assay for certain chemicals (39,42). Dutka and Kwan (43) compared the Microtox assay with three other bacterial screening procedures to assess changes in the toxicity of chemicals in mixtures and found that each toxicity test displayed its own sensitivity pattern. Consequently, they recommended a battery of several toxicity screening tests to assess the presence of toxicants.

One of the most striking examples of the inadequacy of the Microtox as a sole toxicity screening test was reported by McFeeters et al. (34). These workers observed that a dual species assay which included an algal species was over two orders of magnitude more sensitive than the Microtox test for certain herbicides that inhibit photosynthesis. The Microtox bacteria, having no photosynthetic apparatus, would not be affected by such toxic mechanisms.

Finally, the optimum conditions required for the Microtox bacteria may prevent some applications. Factors such as pH and salinity must be within a certain range, and sample colour and turbidity can cause problems. In most cases, adjustments can be made to overcome these difficulties, but there are limits to the validity of these adjustments.

2.2.2 The ATP Assay. Although an effective battery of microbial toxicity screening tests will probably include the Microtox test, at least one or more of the others is likely to involve the firefly luciferase assay for ATP. ATP (adenosine triphosphate) is the chemical through which most of a cell's energy is stored and transmitted (44). It is present in all living cells whether they are prokarytoic, eukarytoic, aerobic or anaerobic. Cellular ATP levels decrease rapidly after death and ATP is not an integral part of non-living systems (45).

Because of these features and since a sample's ATP content is directly proportional to its cellular mass (45), the ATP assay has become widely accepted as a measurement of total living biomass. For example, the test is now included in both the ASTM standards manual for water (46) and in Standard Methods for Examination of Water and Wastewater (10).

The assay is performed by extracting the ATP contained in the microorganism of a sample and mixing the extract with an enzyme reagent known as luciferin-luciferase which has been purified from firefly tails. The enzyme reagent is highly specific for ATP and for every molecule of ATP consumed in the reaction, a photon of light is emitted. This light is then quantified with a photometer. The instrument signal when standardized with a solution prepared from crystalline ATP can be read to yield the amount of ATP in the sample, which in turn is proportional to the amount of living biomass.

The assay is simple, inexpensive, and rapid with the total analysis time requirements usually ranging from several seconds to several minutes. In contrast to Microtox this technique can be used either in the laboratory with standard organisms or as a field procedure with wild organisms. Reagents and moderately priced instruments are commercially available from a variety of manufacturers.

Many users have found that the technique is ideal to assess the impact of toxic substances on existing or endogenous microbial populations. The U.S. Environment Protection Agency has used the assay to monitor the mortality of plankton caused by hot water discharges from thermal generating plants (47). Brezonik et al. demonstrated the effectiveness of the assay in monitoring mercury toxicity to algae (48). Several reports have described the use of ATP to monitor stress and toxic effects in biological waste treatment systems (49,50,51) and a number of industrial institutions and service companies use the assay to evaluate the effectiveness of industrial biocides (52,53,54). In addition, the ATP assay has been used to predict the impact of cadmium (for example released by acid rain) on the bacterial populations in lake waters (55). The assay has also been used in mammalian assays. Munford and Lewtas (56) performed a cytotoxicity test of fly ash particles against rabbit alveolar macrophages using the ATP technique.

In addition to being applicable to any living system, the ATP assay offers another strong advantage: very small amounts of cells can be measured. No other technique is capable of measuring less than 1000 viable bacterial cells in under one minute. This is important from two perspectives. First, it permits smaller amounts of biomass to be used in the rapid screening test. For certain compounds, particularly those whose toxic mechanism involves a reaction with cell surfaces, this increases the sensitivity of the assay. Unfortunately, in the experimental design of environmental studies, consideration of this phenomenon often has been neglected. Second, where adsorption effects of sample containers are minimal, ATP assay systems can be performed in small containers (e.g., microlitre plates). Because only 50 to 100 microlitres of sample are routinely used for analysis, relatively small laboratories could handle large numbers of tests. Also, where only a small volume of test sample is available, no difficulty would be encountered.

Because ATP is part of all living cells a test system containing a significant amount of biomass from cells not relevant to the test must be subjected to contaminant removal by differential filtration, centrifugation, selective extraction or other procedures. Removal of the contaminants may introduce bias and extra labour into the test method.

ATP is typically measured by an enzyme luciferase, and it is possible that the test medium or the toxicant itself may inhibit the luciferase and prevent the measurement of ATP. In many cases, this problem may be easily overcome by sufficient dilution of the ATP extract prior to analysis.

Most investigations using the ATP assay have emphasized the evaluation of the effects of toxic agents on the viability of the total endogenous microbial population of a sample rather than on standard laboratory organisms. A preliminary study recently conducted by Parker and Pribyl (57) examined Enterobacter aerogenes, Escherchia coli, Pseudomonas aeruginosa and Nitrobacter agilis as surrogate organisms for toxicity screening of organic pollutants. They found a good cause and effect relationship between dosage and ATP decrease in the test organism. However, all organisms tested provided inadequate sensitivity. A critical examination of their publication indicates that the experimental protocol may have created problems. Relatively high amounts of biomass appear to have been involved and loss of cell viability was measured by decrease in ATP culture content rather than inhibition of cell growth by inhibition of culture ATP synthesis.

The inhibition of cell growth is more sensitive than loss of viability because growth can be stopped without death occurring. Furthermore, cells often retain significant amounts of ATP in the early stages after death. This phenomenon would also have reduced the sensitivity of the toxicity screening protocol. It is unfortunate that these investigators chose to monitor toxicity by ATP reduction rather than by inhibition of ATP synthesis. The measurement of growth inhibition of a surrogate bioassay organism by the ATP assay has many attractive features.

The general advantages of the ATP assay when applied to measure growth inhibition can be summarized as follows:

1) The technology for ATP assays has been well developed and both reagents and instruments are commercially available from a variety of manufacturers.
2) The test protocol is applicable to any species of microorganism.
3) The sensitivity of the ATP assay is great enough that relatively small quantities of biomass are required for measurement. This reduces artifacts due to biomass adsorption of toxic material and has the potential to increase bioassay sensitivity. In addition, this permits toxicity testing in relatively low nutrient environments.
4) The sensitivity of the ATP assay is sufficient that ATP extracts can be greatly diluted before analysis and still be measured precisely. Assay interference due to

luciferase inhibition can thus be easily eliminated. Furthermore, only small aliquots of sample are required for analysis. This eliminates difficulties which arise when only small amounts of samples are available for toxicity testing.

5) Extraction of ATP in this application of the ATP assay can be accomplished very simply by addition of a sample aliquot to the appropriate acid, solvent, or nucleotide releasing agent.

6) ATP extracts when stored in the appropriate buffer are stable without freezing and so can be analyzed when convenient.

7) The entire procedure or portions of it are easily amenable to automation.

8) Highly specialized training is not required to perform the ATP assays. The level of skill required is similar to that for the Microtox assay. This technique is equally applicable in the laboratory or field.

9) Results are available much sooner than with other growth inhibition tests. With the ATP assay, if exponentionally growing cells are used, accurate results will probably be obtained within one to three generations of growth. For many species of bacteria at 30°C, this would be equivalent to under two hours of incubation.

10) If concentrations of toxicant greater than the minimum to cause growth inhibition are tested, the test could demonstrate whether the toxicant can only inhibit growth or whether it also can kill the cells.

11) It is possible for the same instrumentation used for the ATP assay to perform other types of toxicity tests, including the Microtox test (see Appendix).

The only difficulty still likely to apply in this application of the ATP assay is sample contaminations with unwanted organisms. This is unlikely for very toxic samples. In addition, as for all growth inhibition tests, the appropriate environment must be provided to permit the bioassay organisms to grow. For some chemicals this environment may reduce or eliminate toxicity.

2.2.3 Algal Assays For Toxicity Testing. In most cases, batch algal tests are used, where cultures of algae are prepared and distributed into aliquots which are "challenged" with samples suspected to contain toxic substances. After appropriate incubation periods, the algae are examined for toxic effects using any of a variety of methods. Less frequently, because of increased labour and higher costs, continuous cultures are used to overcome disadvantages of batch tests such as artificially high nutrient requirements, artifacts caused by nutrient exhaustion and catabolic product accumulation, chemical adsorption to the container, pH rises, and oxygen depletion (58).

Because algae are representative of primary production in the food chain, an algal assay is very important for characterization of a sample's potential threat to the environment. As previously discussed, an assay capable of monitoring toxic effects to general cell functions may not identify algicidal and algistatic chemicals which primarily affect photosynthesis. Algal tests may also exhibit superior sensitivity to other types of toxicants. For example, there is evidence that algal assays are more sensitive than aquatic animal assays to complex wastes such as industrial and municipal effluents, while in general the aquatic animal tests have been more sensitive to single pollutants such as pesticides and heavy metals (58,59).

The major limitation in the use of algal assays for toxicity screening is their relatively slow growth rate. For example, during exponential growth, Selenastrium capricornutum, one of the most commonly used species, typically requires over eight hours to double in number, while many bacteria can achieve this in 30 minutes.

Another significant limitation in the use of algal assays is the requirement of maintaining a stock culture. This introduces extra labour which must be carried out even during periods when a toxicity testing program is not being conducted. Furthermore, maintenance of a stock culture requires aseptic culture skills in order to prevent bacterial contamination, which can alter the results of an algal assay.

Other limitations of algal assays depend on the procedures used to evaluate the effects of toxic materials. The most common procedure used to evaluate toxic effects in algae is the measurement of growth inhibition. Most frequently, growth is monitored by the enumeration of cells under a microscope. This procedure has several advantages: it is inexpensive, it allows detection of contamination of the test culture, and it also permits detection of morphological changes in the test culture induced by a toxic agent. However, the procedure is tedious, relatively slow, and error rates are often high (60).

An electronic counter such as the Coulter counter is a relatively fast, precise, and easy way to measure cell growth. However, the instrumentation is relatively expensive and has not been optimized for counting algal cells (60). Difficulties may be encountered because such counters do not differentiate between dead and living cells and debris of similar size to the test culture (61). Neither electronic counters nor direct microscopic analyses are suitable for counting filamentous algae.

Another inexpensive technique to measure algal cell growth is measurement of dry weight. This technique has difficulties similar to microscopic counting methods and in addition suffers from interferences by non-algal particulate matter.

Measurement of cellular constituents such as chlorophyll and ATP are also used. Of these two, measurement of chlorophyll has been more widely used. It has the advantage of being specific for photosynthetic organisms. Furthermore, if assay conditions permit in vivo fluorescence measurements of intact cells, measurement is very simple. On the other hand, the ATP assay can detect smaller quantities of biomass and appears to respond more rapidly to toxic stresses (62). The main disadvantage of the ATP assay for algal toxicity screening is probably the requirement that test cultures be relatively free of contaminants. However, the validity of toxicity screening using contaminated cultures is somewhat dubious.

Another category of measurements frequently used to assess algal assays is based on physiological responses of test organisms. Incorporation of radioactively labelled carbon from $NaH^{14}C0_3$ and measurement of oxygen evolution appear to be most often used parameters of this type. Both these methods can provide rapid results because they do not require significant cell growth before measurement of a toxic insult. However, they have serious drawbacks. The radioactive method requires expensive equipment and presents radioactive materials handling and disposal problems, while the oxygen evaluation tests require the use of dense cultures.

One technique that appears promising is the measurement of DCMU (the herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea) induced fluorescence in chlorophyll a. When cells are not healthy, the induction of fluorescence by DMCU is reduced (63). It has been proposed that this phenomenom could be used to assess toxicity (64). Because the technique appears simple and requires relatively low cell numbers, it will be very interesting to see how it performs with various toxicants.

2.2.4 Dehydrogenase Assays. Dehydrogenases are enzymes that oxidize their substrates by transferring electrons through the electron transport system. Their activity can be measured using dyes such as tetrazolium salts (e.g., TTC, INT), methylene blue, and reazurin, which change colour when reduced. If these dyes are added to an actively respiring culture, the amount of colour produced will be proportional to the extent of dehydrogenase or electron transport activity. A toxic substance that interferes with respiration will reduce the amount of colour. The relative amount of colour production is measured with the aid of a spectrophotometer. Typically, the reduced dye is extracted with a solvent before measurement by spectrophotometer.

The main advantages of dehydrogenase assays is that they are relatively easy to perform and are inexpensive. In addition, the reduction reaction occurs relatively quickly.

The test has been used in a variety of applications. Using reazurin reduction, the toxic effects of arsenical compounds, cyanide, mercury, and phenolic compounds on a mixed bacterial culture were measured (65); with TTC (triphenyl-tetrazolium chloride), toxic effects to activated sludge have been studied (66,67); and, effects of inorganic and organic toxicants on biological purification systems have been compared (68).

A serious limitation of dehydrogenase assays is the requirement for relatively large amounts of cells for the test procedure. This, as discussed previously, tends to reduce the sensitivity of assay for toxicity screening. The cells could be concentrated prior to dehydrogenase measurements but this increases the labour cost of the assay.

A dehydrogenase assay which recently has become widely used involves microscopic examination of cells which have been incubated with a tetrazolium salt (usually INT, 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride) (69). When the tetrazolium salt is reduced by dehydrogenase activity, it forms a coloured precipitate (formazan) which is trapped inside the cell. With microscopic examination, respiring cells are identified. It is possible to examine the respiration of individual species in a mixed population if that species has unusual morphological characteristics or if it can be selectively stained (e.g., with fluorescent antibody stains, known as the FAINT technique) (70). This technique has been used to measure the toxicity of mercury to algal cells (71) and the toxicity of chlorine to filamentous bacteria in activated sludge (72).

Although this variation of the dehydrogenase assay is tedious and somewhat slow for screening large numbers of samples, it appears to have considerable merit for specific applications and as a test in a second tier of evaluation of toxic impacts on individual species of a microbial community.

2.2.5 Growth Inhibition Assays. In growth inhibition assays, cultures of microorganisms are prepared and allowed to grow in the presence and absence of toxic substances. After appropriate exposure time (31) to the toxicants, the amount of growth in the test cultures is measured.

Because all basic cellular activities must be functioning properly for good growth, a wider range of toxic mechanisms can be detected than in those assays that focus on only one cellular function. For example, Alsop et al. (73) suggested that respiration can continue for some time even when growth has been inhibited.

On the other hand, as for all growth assays, the testing environment is restricted to one that supports active growth. Thus, some aspect of the growth environment may neutralize the toxic components to be tested.

Other advantages and limitations of these assays depend on the method used to measure growth. The most common methods to measure growth are measurement of culture turbidity and plate counts. Turbidity measurement is quick, simple, and inexpensive. A protocol and preliminary investigation has been described by Allsop et al. (73) for predicting toxic effects on biological waste treatment systems. In this procedure, sewage microorganisms are grown in a medium containing acetate and nutrient broth and incubated in the presence of various dilutions of toxic test materials. Cultures containing no toxic "challenge" serve as controls and growth is compared after 16 hours to calculate the concentration causing a 50 percent growth inhibition.

While very simple, turbidity measurement has a major limitation in that dense cultures must be used. A test similar to that described by Alsop et al. was studied by King (74) and was compared with Microtox and several other microbial toxicity tests against eight toxic compounds. The Microtox assay was significantly more sensitive than the other assays in all cases.

The use of plate counts to measure inhibition can overcome the problem of the dense culture requirement. However, the plate count is a more difficult test and requires incubation periods too long for practical rapid screening tests. These difficulties could be overcome by using the ATP assay.

2.2.6 Measurement of Inhibition of Oxygen Uptake. Many microorganisms use oxygen as the final electron acceptor during respiration. If a toxic material inhibits respiration, the microorganisms cease to consume oxygen. This can be measured by devices such as the Warburg respirometer or by measuring the dissolved oxygen (DO) in the medium of the microorganisms by means of an oxygen electrode.

Measurement of oxygen consumption by respirometers is time-consuming and requires relatively expensive equipment. Oxygen electrodes are more convenient and less expensive. The equipment is standard apparatus at most waste treatment laboratories. Generally, the rate of oxygen consumption is compared for microbial populations in the presence and absence of a toxicant after appropriate periods of exposure (75,76).

A variation of this test is the use of the five-day BOD assay to measure toxicity (77). This test is valuable in providing information on the ability of microorganisms to acclimatize to a toxic material . However, it is too slow to be considered for the first tier of a battery of toxicity screening tests. In addition, the range of concentrations producing a quantifiable effect is often smaller than for other methods such as Microtox and growth inhibition (78).

The major problem with toxicity testing using oxygen uptake measurements is that large amounts of cells are required to produce a measurable consumption. With this in mind, it is interesting to note that in a comparison of oxygen uptake rate using a synthetic activated sludge against three other bacterial toxicity tests (Microtox, a motilily test using Spirillum volutans, and growth inhibition of Pseudomonas fluorescens), the oxygen uptake test appeared to be the least sensitive test more frequently than any other test (39). The other major limitation of this type of test is that the test environment must be compatible with oxygen.

2.2.7 Microcalorimetry. Although high equipment costs make this technique inaccessible to many laboratories, some mention of microcalorimetry should be made. In this technique, heat produced during microbial metabolism is measured.

The technique, when applied for toxicity testing, has many attractive aspects. For example analyses are non-invasive and non-destructive and can be applied to any species of microorganism. Recently, a commercially available flow cell microcalorimeter was described by Beaubien and Jolicoeur (79) to measure the toxicity of metals, alcohols, and surfactants to a laboratory activated sludge process. Their technique could monitor relatively low cell concentrations (limit of approximately 10^4 cells per mL), exhibited a fast response time of approximately 60 seconds, and had potential to be automated.

2.3 Microbial Toxicity Screening for Characterization of PCF Power Station Combustion Wastes

As discussed in previous sections, the use of a battery of tests rather than a single one is very important for preliminary characterization of any waste. It is impractical to develop a completely comprehensive toxicity screening program capable of detecting every possible toxic hazard, but the battery approach does reduce the risk of an incomplete or incorrect assessment.

In addition to providing an accurate indication of potential toxicity hazards, an ideal screening test would have the following characteristics:

a) No requirement for special skills.
b) Simple and easy to perform.
c) Results available in the same day.
d) Test organisms that are not site-specific so that laboratories in different geographical locations could perform the same test.
e) No proprietory aspects or ones requiring extensive development.
f) Sufficiently low cost that extensive testing is feasible.

At this time, the only bio-assay test that fills all these criteria well is the Microtox assay. No other microbial test has been described which does not require cultivation of the assay microorganism. This task is not difficult but usually requires an awareness and attitude that comes from training in microbiology.

Many studies at engineering laboratories use activated sludge as an innoculum. Such innocula, while relatively similar from plant to plant, do have site-specific variations which will always introduce an unknown variable into a test protocol. The difficulty could be overcome if appropriate preserved cultures and test media were commercially available. Although the American Type Culture Collection does offer desirable cultures, their high cost is prohibitive for single-use experiments. Using such cultures offers some degree of standardization but generally necessitates some cultivation work to provide a low source of microoganisms. For quality control, cultures that have been freeze-dried in water-soluble discs are also available, but the organisms from this source tend to be for clinical applications.

One exception was located. The Polybac Corporation manufactures a product called Polyseed™ which consists of capsules containing several microbial species which have been lyophilized for use as a seed in BOD (biochemical oxygen demand) tests. To prepare the organisms for use, the contents of the capsule are added to the appropriate medium (e.g., BOD dilution water) and incubated for several hours with aeration.

Intended for use in BOD analyses, the Polyseed organisms were considered more relevant to this project than clinical organisms. Because of this, their ease of preparation, and their commercial availability, they were selected for use in the second screening procedure of the test battery.

Because of the speed, simplicity, and sensitivity of the ATP assay in addition to other attractive features when used to monitor growth inhibition, this analytical technique was chosen to monitor the effects of test samples on the Polyseed organisms.

As the final member of the test battery, it was decided that an algal assay would be chosen due to the importance of algae as primary producers. Although it is normally desirable to test a variety of algal species because different species have greater sensitivities to different toxicants (73), the limited scope of this project only permitted the selection of a single species. Selenastrum capricornutum Printz was chosen because it is a recommended species for freshwater (10). It has many ideal properties for toxicity testing including an easily identifiable morphology facilitating detection of cultures contaminated with other algal species, and a tendency to remain easily dispersed.

Researchers have selected this organism for toxicity testing of a wide variety of compounds and samples, including mercury (48), zinc sulphides (80), aromatic compounds from coal conversion (81), manganese, copper, and lead (82), crude oil and furnace oil (83), urban snowmelt water (84), industrial wastes (85), and an aqueous extract from a scrubber ash slurry generated by a coal fired power plant in Montana (86).

Selenastrum capricornutum can be obtained from The American Type Culture Collection but unfortunately is not available, like Polyseed, in a form appropriate for single applications. Because of the desirability of including an algal assay, this disadvantage was overlooked. Again, the ATP assay was chosen to measure the inhibition of the organisms growth. Because of the technique's speed and sensitivity, it was felt that it offered the best practical chance to obtain results within the same day.

3 ANALYTICAL METHODS

This section of the report describes sources and methods of sampling the coal combustion residues, extraction procedures, organohalide characterization, inorganic chemical characterization, toxicity screening procedures, and quality control procedures used during the study.

3.1 Coal Combustion Residue Sampling

Table 3 describes the sources of coal combustion residues characterized during the study. These sites were selected to provide a broad cross-section of pulverized coal fired (PCF) combustion wastes generated in Canada. Wastes from different combustion methods (pulverized coal and atmospheric fluidized bed), and coal types (high and low sulphur, bituminous, sub-bituminous, lignite and coal rejects), and different waste types (fly ash, bottom ash, bed material and baghouse material) were obtained. Triplicate samples of the fly ashes were obtained to provide an indication of variation of the waste characteristics at a given site.

Fly ash samples were obtained from ash storage hoppers below the gas cleaning equipment. Samples were collected dry and in a manner that minimized contact with the atmosphere. Fluidized bed combustion (FBC) samples were taken from storage hoppers adjacent to the atmospheric fluidized bed combustor (AFBC) unit. Samples were collected dry. PCF bottom ash samples were collected wet; they were in contact with the ash sluice water for an unknown period under unknown conditions prior to sampling. Sluice water samples were obtained from the surge tank in the case of Dalhousie Generating Station (G.S.) while at Poplar River G.S. they were taken from the ash sluice water return lines from the ash lagoons. These ash-contacted wastewaters were expected to be representative of eastern and western Canadian operating conditions. They were included in the chemical characterization and toxicity screening so that laboratory generated leachates could be compared to those produced in ash systems at operating generating stations.

National Bureau of Standards (NBS) standard fly ash No. 1633a was also included as one of the coal combustion wastes in the sampling program. This ash served as a reference point so that the results of this study could be linked to other investigations. All samples were stored in air-tight containers until the in-laboratory extraction and characterization studies were begun.

TABLE 3 SOURCES AND DESCRIPTION OF SAMPLES CHARACTERIZED

Site/ Operator	Method of Combustion*	Description	Residue/ Wastewater
Summerside PEI/ Dept. National Defence	AFBC	Commercial scale AFBC using high sulphur eastern Canadian bituminous coal	bed material; baghouse material
Dalhousie G.S./ New Brunswick Power	PCF	215 MW generating station burning high sulphur bituminous coal; equipped with an electrostatic precipitator; recirculating sluice water from the botton ash system	bottom ash; fly ash; sluice water
Lakeview G.S./ Ontario Hydro	PCF	2400 MW generating station burning medium sulphur bituminous coal; equipped with electrostatic precipitator	bottom ash; fly ash
Poplar River G.S./ Saskatchewan Power Corp.	PCF	600 MW generating station burning low sulphur lignite coal; equipped with an electrostatic precipitator lagoon water from the recirculated combined ash lagoons	bottom ash; fly ash; ash lagoon
Milner G.S./ Alberta Power	PCF	115 MW generating station burning bituminous coal, coal rejects and natural gas, equipped with a baghouse	bottom ash; fly ash;
Battle River G.S./ Alberta Power Limited	PCF	737 MW generating station burning sub-bituminous coal; equipped with with an electrostatic precipitator	bottom ash; fly ash
NBS Standard	PCF	standard reference coal fly ash	fly ash

* AFBC - Atmospheric Fluidized Bed Combustor.
PCF - Pulverized Coal Fired Combustor.

During the extraction and characterization, the origin and type of sample were not known by the analysts. Replicate samples were also distributed randomly during the laboratory program to minimize any potential bias.

3.2 Extraction Procedures

The coal combustion residue samples were split with one portion subjected to an aqueous extraction while the other underwent solvent extraction.

Aqueous Extraction. While some life forms can exist in a suspended state in a non-aqueous environment, all organisms require water to function actively. Characterization of combustion residues with regard to environmental hazards must consider the water soluble components leachable from the wastes.

An aqueous extraction procedure developed by the Wastewater Technology Centre of Environment Canada (87) was utilized. It involves contacting the waste in a 4:1 liquid-to-solid ratio for 24 hours using de-ionized water. During this period, the mixture is radially rotated at 10 rpm in sealed plastic bottles with approximately 20 percent headspace. Following the extraction, samples are allowed to settle for approximately one hour. The resulting supernatant is then vacuum filtered through 0.45 µm pore size filter paper. Samples were stored in head space free amber glass bottles at 4°C until analyses were conducted.

Solvent Extraction. To compare the amount of potentially leachable organic halides present in the combustion residues and the amount that is leached under the conditions of the Wastewater Technology Centre procedure, a more rigorous solvent extraction was performed on a limited number of representative samples. As mentioned in the literature review, solvent extractions have typically been used by other researchers to characterize organohalides in solid wastes.

The solvent extraction procedure chosen for the combustion wastes was taken from Coburn et al (28). Ten grams of sample were extracted with 80 mL of I N HCl for two hours on a wrist-action shaker. The extract was vacuum filtered through an organic-free glass fibre disc, followed by three 50-mL washings with organic-free water.

The retained solids and filter were allowed to air dry in a fume hood. The filtrate and washings were sequentially extracted in a Teflon separatory funnel with a total of 150 mL toluene. The toluene extracts were combined in a 250-mL boiling flask and subsequently used to extract the dried filter and retentate on a soxhlet. The sample was extracted for 24 hours at a cycle rate of four times per hour.

After passing through sodium sulphate, the toluene extract was concentrated to slightly less than 1 mL and made up to 1 mL with toluene.

3.3 Inorganic Chemical Characterization

The aqueous extracts were subsequently chemically characterized. Table 4 summarizes the analytical methods, which included wet chemistry, instrumental techniques, ion chromatography, induced coupled argon plasma spectroscopy and atomic absorption. Typically standard methods (10) were used.

TABLE 4 ANALYTICAL METHODS USED FOR INORGANIC CHEMICAL CHARACTERIZATION

METHOD	ANALYSIS
Wet Chemistry/Instrument	pH, total dissolved solids, alkalinity
Ion Chromatograph	chloride, fluoride, nitrate, nitrite, phosphate
Inductively Coupled Argon Plasma Emission	silver, aluminum, beryllium, boron, calcium, cadmium, cobalt, chromium, copper, iron, potassium, magnesium, manganese, molybdenum, sodium, nickel, phosphorus, lead, strontium, thorium, titanium, vanadium, zinc, zirconium
Atomic Absorption	arsenic

3.4 Total Organic Carbon (TOC), Total Organic Halide (TOX) and Purgeable Organic Halide (POX) Analyses

Total organic carbon (TOC) analysis was performed by at least triplicate injections using combustion-infrared analysis on a Beckman Model 915A Carbon Analyser and Model 865 Infrared Analyzer.

A 20-mL aliquot of leachate was acidified to pH less than 2 with HNO_3 and briefly sparged with nitrogen to remove inorganic carbon. The acidified and nitrogen sparged aliquots were analyzed for non-purgeable organic carbon using combustion - infrared, by injection of 40 µL aliquots into the instrument.

For the POX and TOX analyses, each sample received was conditioned and analyzed as follows:

- 500 mL were placed in a volumetric flask.
- 1.0 mL of 0.1 molar sodium thiosulphate solution was added.
- 10-mL aliquots of the above were removed with a syringe and transferred to the POX analyser chamber and analysed for purgeable organic halide content (POX). This was carried out by sparging with carbon dioxide at 45-50°C and combustion of the vapours in oxygen at 800°C, followed by microcoulometric silver ion titrations

of the hydrogen halides formed. These analyses were performed in at least duplicate injections.

- The remaining liquid in the volumetric flask was acidified using nitric acid (high purity) to approximately pH 2. Aliquots (normally 100 mL) of the acidified liquid were processed for total organic halide (TOX) at least in duplicate, by absorption of organics from water onto granular activated carbon (GAC) packed in glass micro-columns and subsequent desorption of inorganic halides by washing the GAC columns with nitrate solution, and combustion of sorbed organics along with the GAC at 800°C in oxygen and immediate microcoulometric silver ion titration of the hydrogen halides formed via combustion.

The TOX/POX analyzer used was the Dohrmann DX-20 Total Organic Halide Analyzer System. It comprises an AD-Z adsorption module and a microcoulometric analyzer MC-1 fitted with a T-620 titration cell. The range, expressed in µg/L as Cl, for the instrument is shown below. This was used to determine the volume of sample required for analysis.

Optimum Range	Workable Range	Sample Volume Passed (mL)
10 - 250	5 - 500	100
20 - 500	10 - 1000	50
40 -1000	20 - 2000	25

The precision of the method is $\pm$ 2 µg/L as Cl ($\pm$ 6 x 10^2 µ moles/L) or +2 percent, whichever is greater. This is equivalent to one standard deviation by instrumental contribution. The technique has a 95 to 100 percent recovery relative to Coulomb's law if all organic halides are adsorbed on the granular activated carbon (GAC) and all trihalomethanes and other organic halides are purged from the sample (POX). The calculations for POX and TOX concentrations are shown below:

POX - the purgeable organic halide concentration in µg Cl^-/L from 10-cm^3 injected samples is: instrument reading x 100.

TOX - $(\mu g\ Cl^-/L) = (C_1 + C_2 - 2C_3)\ 1000/V$

where: C_1 = halide content of 1st (upper) GAC column µg Cl^-)

C_2 = halide content of 2nd (lower) GAC column µg Cl^-)

C_3 = halide content of blank GAC column after passing 100 mL of "blank" water through and washing with 2 mL nitrate wash solution. Our value of C_3 is 0.45 (i.e., $2C_3 = 0.90$).

V = volume of sample processed (mL)

For the analysis of solvent extracts of the coal combustion residues for organohalides, five 10-µL aliquots of the toluene extract were directly injected into the boat of the Dohrmann Model MC-20 microcoulometer under the same conditions as the GAC from the leachate TOX analysis.

3.5 Toxicity Assessment: Materials and Methods

3.5.1 Microtox™ Assay. One millilitre of Microtox reconstitution solution was placed into a Microtox cuvette in a cooling block kept at approximately 4°C. After 15 minutes, a vial of Microtox reagent was removed from a 4°C refrigerator and without hesitation, the vial was opened and the reconstitution solution was quickly tipped from the cuvette into the vial. The resulting suspension was then swirled and poured back into the cuvette which was returned to the 4°C cooling block. The suspension was mixed by pipetting up and down 20 times with a Clay Adams Selectapet set at 0.5 mL. The reagent was now ready for use.

Analyses were conducted using Microtox reagents and cuvettes (Beckman Instruments Inc.). Measurement of light from the Microtox reagent was performed using a Turner Designs Model 20 Photometer connected to a recirculating water bath (Lauda Model RM3, Brinkman Instruments) to maintain the photometer reaction chamber at 15°C.

Prior to analysis, each sample was adjusted to contain 2.0 percent sodium chloride by addition of a 22 percent NaCl solution. A dilution series for the sample was then prepared in 2 percent NaCl. The series was prepared in glass cuvettes and held in a cooling block at 15°C.

A series of ten cuvettes, each containing 500 µl of a suspension of Microtox bacteria was prepared from a stock suspension maintained at 4°C. This suspension had been prepared by dispersing 500 mL of Microtox diluent into 10 cuvettes and then adding 10 µL Microtox bacteria stock suspension. These were subsequently mixed by pipetting the suspension up and down five times with a 250-mL pipette.

After these cuvettes had been held in the cooling block for 15 minutes, the light emitted from each cuvette was measured. At timed intervals, 500 microlitres from each sample dilution was added to a cuvette containing the Microtox bacteria and mixed. This procedure was performed in duplicate. The light emitted from the bacteria was measured after 5 and 15 minutes exposure to the sample.

The test was repeated examining only the 15-minute exposure. EC_{50} values for these exposure periods were estimated by graphically determining the percentage of sample which caused a 50 percent reduction of light relative to a control which received 500 µL of 2 percent NaCl instead of sample.

3.5.2 Polyseed™ Assay. Prior to initiating the toxicity assay, the contents of one Polyseed capsule (Polybac Corporation) were added to a 250-mL erylenmeyer flask containing 50 mL of BOD dilution water (10) that had been supplemented with 15 mg/L glucose and 15 mg/L glutamic acid. The flask was incubated in a controlled environmental shaker (Model G24 New Brunswick Scientific Co. Ltd.) set at 250 rpm and 30°C for four hours.

It was found that this was the incubation period before a logarithmic increase in biomass according to the ATP assay was observed. At the end of this period, five morphologically different types of microorganisms were observed. Most cells appeared to be actively respiring according to a microscopic examination following a 10-minute incubation with INT (see Section 2.2.4).

Before "challenging" the microorganisms in a toxic test, it was necessary to dilute the Polyseed suspension at least 100-fold to provide a desirable level of biomass. To prevent clumps of microorganisms from being used in the test, the Polyseed suspension was allowed to stand unagitated for two minutes before an aliquot for dilution was removed from the top portion of the culture. It was also found that logarithmic growth following the dilution did not occur unless the glucose-glutamate-BOD medium was supplemented with 10 percent (volume/volume) of filter sterilized liquor obtained from a previous Polyseed suspension. For convenience, the filter sterilized liquor was stored frozen in 5-mL aliquots which were thawed just prior to use. Presumably, the filtrate contained growth factors absent in the glucose-glutamate-BOD medium. By weighing a Polyseed capsule before and after addition of its contents to the 50 mL of glucose-glutamate-BOD medium, it was calculated that the maximum amount of this material that would be present in the toxicity assay would be 190 mg dry weight per litre, a moderately low concentration.

After diluting the Polyseed culture to one to two nanograms ATP per mL as described above, the diluted suspension was drawn and expelled seven times in a 50-mL combi-tip attached to an Eppendorf repeater pipette to disperse the microorganisms thoroughly. The resulting suspension was drawn up a final time and à series of 2-mL aliquots were rapidly dispensed into 20-mL glass scintillation vials. The vials were placed into a rack attached to the clips on the controlled environmental shaker set at 30°C and 250 rpm and allowed to incubate for 25 minutes.

During incubation, a dilution series of leachate sample in glucose-glutamate-BOD medium was prepared in duplicate. Glucose-glutamate-BOD medium without added sample was used as a control. For dilution series having a primary dilution greater than 10 percent, the primary dilution was prepared in autoclaved water which had been purified by a Milli-Q system (Millipore Corporation) and the nutrient content was adjusted by addition of a solution containing a 10-fold concentration of the glucose-glutamate-BOD medium.

At the completion of the 25-minute equilibration period, 0.400 mL of the Polyseed suspension was removed from each scintillation vial for ATP analysis. To the remaining 1.6 mL in each vial, an equal volume from a member of the leachate dilution series was added. The vials were returned to the shaker and removed again for ATP analyses after 1 h and 2 h of incubation.

IC_{50} values for these exposure periods were then estimated by graphically determining the percentage of sample which caused a 50 percent reduction of growth as indicated by culture ATP level relative to the control.

3.5.3 Selenastrum capricornutum Assay. A Selenastrum capricornutum Printz culture was obtained which had been derived from an American Type Culture Collection. To serve as a stock culture for toxicity testing, the organism was grown in 250-mL erylenmeyer flasks filled to 50 mL using a synthetic algal culture medium recommended in Standard Methods for Examination of Water and Wastewater (10).

Illumination was provided by "cool white" fluorescent bulbs with approximately 4000 lux at the liquid level of algal culture. A water bath set at 30°C was used to control the temperature of the growth environment. Although this temperature is slightly higher than recommended by the procedure, it is closer to the temperature that was used in the toxicity testing procedure.

To maintain the algae in an exponential growth phase, the culture was diluted daily with algal medium so that the ATP level of the culture never exceeded 30 ng/mL.

To prevent bacterial contamination, all nutrient additions and cell transfers were performed using aseptic techniques in a biological safety cabinet having laminar air flow. The stock cultures were examined microscopically throughout the testing program and no bacterial contamination was observed.

Since a practical screening procedure should provide an indication of toxicity within the same day, conditions for this test procedure were chosen to maximize growth rate. Kean (65) demonstrated that providing a relatively high illumination intensity significantly improved the growth rate of Selenastrum capricornutum. In addition, it was felt that the warming effect of the light bulbs probably also improved the growth rate. The algal assay was conducted in 20-mL glass scintillation vials which were placed between two 1.2-m 35-watt "cool white" fluorescent light bulbs. Small plastic discs were glued to the floor of the light fixture to act as guides to ensure that vials would be placed in a reproducible manner from one test to the next.

In this test apparatus, the ATP content of the culture doubled in approximately 6 hours and increased approximately five-fold in 24 hours. In contrast, in the lower light intensity environment used for maintaining the stock culture, it took almost 24 hours for the ATP levels of the culture to double.

The reproducibility of the culture growth over 24 hours in the test environment was adequate but further improvement would have been desirable and appears feasible. Two high-priority approaches to improve the reproducibility of culture growth would be 1) optimization of the distance between the fluorescent bulbs and the cultures, and 2) the use of a light diffusing material to cover the fluorescent bulb and minimize variations in light output along the bulb. It may also be possible to further optimize the test method for metal toxicity testing by reducing the chelating agent (EDTA) in the synthetic algal medium. EDTA was retained in the media formulation in this study to ensure rapid algal growth. In addition, it was felt that significant levels of toxic metals would be detected by the Microtox assay.

Prior to initiating the assay, the ATP level in the stock culture was measured. An aliquot of the stock culture was then removed and diluted in synthetic algae medium so that it would contain 1 to 2 ng of ATP per millilitre (approximately 10^4 cells per mL).

The diluted suspension was drawn and expelled seven times in a 50-mL Combitip attached to an Eppendorf repeater pipette to disperse the cells thoroughly. The resulting suspension was drawn up a final time and a series of 2-mL aliquots were rapidly dispersed into 20-mL scintillation vials. The vials were then placed in the test illumination apparatus and allowed to equilibrate for 25 minutes.

During incubation, a dilution series of a leachate sample was prepared in duplicate using synthetic algal medium as a diluent. Algal medium without sample was used as a control. For dilution series having a primary dilution greater than 10 percent, the primary dilution was prepared in autoclaved water purified in a Milli Q system and the nutrient content was adjusted by addition of a solution containing a 10-fold concentration of the synthetic algal medium.

At the completion of the 25-minute equilibration period, 0.400 mL of the algal suspension was removed from the scintillation vial for ATP analysis. To the remaining 1.6 mL in each vial, an equal volume from a member of the dilution series was added. The vials were returned to the test illumination apparatus and removed again for ATP analyses after 6 and 24 hours of incubation.

IC_{50} values for these exposure periods were then estimated by graphically determining the percentage of sample that caused a 50 percent reduction of growth as indicated by culture ATP levels relative to the control.

ATP extracts of the assay cultures and their stock cultures were prepared by injecting 0.400 mL of culture into 0.500 mL of DMSO and mixing. After one to five minutes, 3.0 mL of 0.005 M Na_3PO_4 containing 0.005 M MgEDTA were added and also mixed. Use of the inexpensive Eppendorf repeater pipette for dispensing the DMSO and Na_3PO_4 buffer greatly facilitated this portion of the assay. Although the ATP extracts were now ready for analysis, they were stabilized at room temperature for at least 24 hours.

To measure the ATP concentration in the extracts, 50 µL of the extract were mixed with 50 µL luciferase reagent (Turner Designs Limited) which had been dispensed with an Eppendorf repeater pipette into an 8 mm x 50 mm polypropylene cuvette (Evergreen Scientific Limited). The resulting light was measured in a Turner Designs Model 20 photometer set with a delay of 5 seconds and an integration period of 10 seconds. The luciferase reagent was calibrated every 60 minutes with ATP standard solution (Turner Designs Limited) which had been diluted to 1.0 ng/mL in Na_3PO_4/MgEDTA buffer so that the ATP concentration in the extracts could be calculated from the instrument readings.

As a check for inhibitors in the ATP extracts, an aliquot of the extract from the primary dilution in the test series was diluted with an equal volume of the ATP standard used to calculate the reagent. The ATP from the resulting mixture was measured. In all cases, inhibition of the luciferase was negligible.

3.6 Quality Control

3.6.1 TOX and POX Analyses. Two carbon columns in series of approximately 40 mg granular carbon (GAC), were prepared for each TOX analysis as described in the Dohrmann manual for the instrument. "Blank" values for the carbon columns were determined by passing 100 mL of acidified (to approximately pH 2) deionized water from a Millipore deionization system (consisting of a carbon column and two ion exchange resin columns) through a pair of GAC columns. The columns were then analysed for TOX by combustion at 800°C in oxygen, followed by microcoulometric silver ion titration. Only the values of the lower column analyses were taken as the carbon blank values since the deionized water might contain traces of organic halides. Values obtained ranged from 0.32 µg chloride to 0.49 µg chloride with an average of 0.45 µg chloride based on eight samples.

To standardize the TOX columns, a 10 000 µg/L chlorine standard of 2, 4, 5 - trichlorophenol (TCP) in methanol was prepared. From this standard, standards of 50, 250 and 500 µg/L TCP were prepared by dilution. Direct injections of the 10 000 µg/L standard onto the analyser boat gave a value of 8 880 µg/L chloride. This indicated the TCP had an 88.8% purity. Aliquots of 100 cm^3 of the 50, 250 and 500 µg/L standards were passed through GAC columns and analysed for TOX recovery. The 50-µg/L standard (actual 44.4 µg/L) gave 48.7 µg/L chloride recovery (110%); the 250-µg/L standard (actual 222 µg/L) gave 211.3 µg/L chloride recovery (95.1%); and the 500-µg/L (actual 444 µg/L) gave 458 µg/L chloride recovery (103%) of the TCP. This is shown on Figure 1. Standards spiked into a background of humic acid (approximately 5000 µg/L TOC) gave the same recoveries.

A sample of pentachloropheno! prepared as a toxicity standard gave almost 100 percent recovery when injected directly and analysed. This method of sample injection was used for the total extractable organic halide analyses. The actual species present in the aqueous leachates may be quite different from the standard and the recoveries may be quite variable for each organic halide present. The Dohrmann instrument manual, however, claims 95% to 100% recovery of all organic halides adsorbable on the GAC as trihalomethanes.

Standards for purgeable organic halide analysis were prepared by dissolving or mixing chloroform, bromoform and bromotrichloromethane in methanol (separately). For analysis, 2 µg/L aliquots of each standard were injected into 10 mL of blank water and injected into the POX analysis chamber where the purgeable organics were swept by a

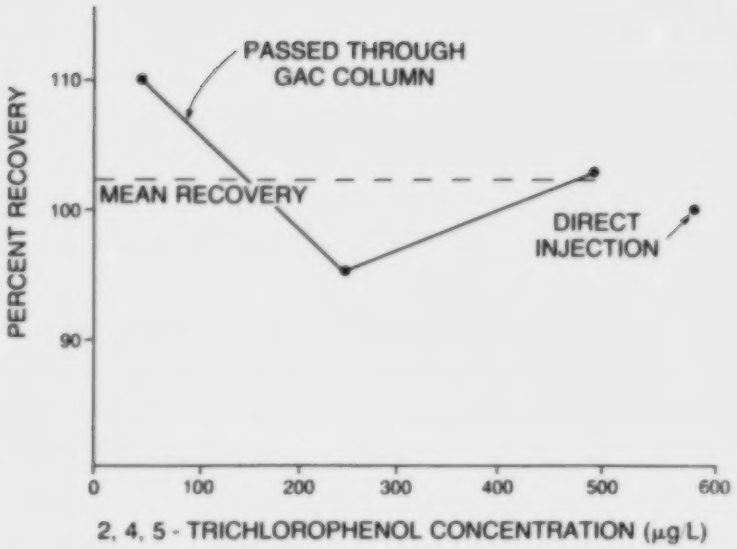

FIGURE 1 RECOVERY OF STANDARD ORGANOHALIDE

stream of carbon dioxide gas at 45-50°C and combusted with oxygen at 800°C in the furnace prior to silver ion microcoulometric titration. The chloroform recovery was 117%; the bromoform recovery was 101%; and the bromodichloromethane recovery was 99.6%. Brominated and brominated-chlorinated compounds are measured on the instrument as the chloride equivalent of the bromide present. The POX method gives excellent repeatability as it is not sensitive to the interference of the TOX methodology. However, as has been noted with two of the aqueous extract samples, sulphide in the sample appears to cause serious interference. The degree of interference caused by sulphide has not yet been established.

The instrument manufacturer claims a 50 000:1 discrimination between inorganic and organic halide species for the TOX analysis. It was found that 3500 mg/L NaCl and 7900 mg/L sodium bromide of aqueous extract sample give serious interference (the sodium chloride gave 30 µg/L chloride and the sodium bromide 17 µg/L chloride readings). Washing the columns with twice as much nitrate reduced, but did not eliminate, the inorganic interference.

Solutions of 0.1 N NaCl 0.1 N NaBr and 0.05 N H_2SO_4 were spiked at 50 µg/L TOX level. Unexpected positive errors of 51%, 43% and 70%, respectively, were found.

Three of the samples were analysed in duplicate. Two full method blanks and two levels of 2,4, 5-trichlorophenol recoveries were run. Three levels of spiked recoveries were also run. The organohalide spikes in methanol were made directly into the initial shaking flask or onto the sample using a total volume of less than 50 µL. The methanol was allowed to evaporate and/or equilibrate overnight prior to commencing acid extraction. The three samples analysed in duplicate and spiked were selected because of their different physical characteristics. The results are shown in Table 5.

TABLE 5 RECOVERIES OF SPIKED SOLVENT EXTRACTS IN TOXICITY SCREENING TESTS

Background	Spiking Level* (µg Cl^-)	Detected (µg Cl^-/mL)	Sample Background (g)	Percent Recovery
-	444	364 ± 10	-	82.0
-	888	648 ± 4	-	72.9
Dalhousie G.S. Fly Ash	444	375 ± 10	12.50	80.6
Summerside Bed Material	222	131 ± 10	9.80	59.1
Lakeview G.S. Bottom Ash	888	754 ± 8	11.63	84.9

* All spikes were Cl^- from 2, 4, 5 - trichlorophenol.

3.6.2 Toxicity Screening Tests. It is important that the test procedure respond in a reproducible manner when "challenged" with a non-toxic environment. This permits an accurate discrimination of toxic effects.

Each member of the test battery of toxicity screening bioassays was examined over the incubation periods used to characterize the leachates in the test program. However, instead of adding various diluents of leachate, only dilution medium was added to the series of vials containing the test organism. Ten replicates of the Microtox bacteria were tested with 10 aliquots of Microtox diluent; 10 replicates of the Polyseed culture were tested with aliquots of BOD diluent supplemented with glucose and glutamate; and 10 replicates of Selenastrum capricornutum culture were tested with aliquots of algal assay medium. Light decay was measured for the Microtox bacteria, while growth (ATP content) was measured for the other two organisms.

The variability of the test was calculated in terms of the coefficient of variation at each time interval utilized in the bioassay procedures. The results have been summarized in Table 6.

The reproducibility of the response of the Microtox bacteria was the best of these assays. The Polyseed test was also very good, showing greatest variation after two hours of incubation with a coefficient of variation of 5.31 percent. The highest coefficient of variation, 14.9 percent, occurred with Selenastrum capricornutum after 24 hours of incubation. This is higher than desirable but just adequate to provide a measurement of growth inhibition. The coefficient of variation for ATP measurements before incubation of the algae had begun, at "0" hours incubation, was only 3.71 percent, a quite acceptable degree of precision. It appears the source of variation in the 6-hour and 24-hour periods was probably due to variations in the growth environment of the algae. Optimization of the growth environment of the algal assay to a further extent appears possible.

In positive control tests, the test battery was challenged with three chlorinated organic compounds with known toxic properties: simazine (a herbicide that inhibits photosynthesis), Kathon 886 and pentachlorophenol (two industrial biocides with different modes of action). The results are summarized in Table 7. Particularly noteworthy is the relative lack of response of the two bacterial tests to the herbicide, simazine. This confirms the work of McFeeters et al (34). The IC_{50} of the S. capricornutum assay was 1.2 mg/L for 6 hours exposure and 0.78 mg/L for 24 hours exposure. This is similar to the algal response in the Tchan assay performed by McFeeters et al., who obtained results of 0.76 mg/L for a 1-hour test and 1.1 mg/L for a 24-hour response.

The Microtox 15-minute EC_{50} value for pentachlorophenol of 1.2 mg/L is the same as reported by King (74), while the Microtox EC_{50} values for Kathon 886 in this project are similar to the findings of Mallak and Brunker (88). These researchers reported a 5-minute EC_{50} value for Kathon 886 of 0.09 mg/L and a 15-minute EC_{50} value of 0.05 mg/L, while results from this study were 0.11 mg/L for the 5-minute EC_{50} and 0.050 mg/L for the 15-minute EC_{50}.

The IC_{50} values for pentachlorophenol and Kathon 886 were similar to the EC_{50} values. This was somewhat surprising because in comparison studies, the Microtox test is often significantly more sensitive than other microbial screening tests.

TABLE 6 REPRODUCIBILITY OF LIGHT MEASUREMENTS OR ATP SYNTHESIS DURING INCUBATION PERIODS OF MICROBIAL BIOASSAY SCREENING TESTS

	Incubation Period								
	Microtox (Light Decay)			Polyseed (ATP Synthesis)			S. Capricornutum (ATP Synthesis)		
	0 MIN	5 MIN	15 MIN	0 MIN	60 MIN	120 MIN	0 HOUR	6 HOUR	24 HOUR
Coefficient of Variation (Standard Deviation/Mean) For 10 Test Vials Innoculated with Control Diluent Instead of Toxic Sample	2.04%	1.78%	2.02%	4.29%	4.37%	5.31%	3.71%	10.1%	14.9%

TABLE 7 RESPONSE OF MICROBIAL TOXICITY SCREENING TESTS TO KNOWN TOXIC COMPOUNDS

Toxic Compounds	5-minute Microtox EC_{50} (mg/L Active Ingredients)	15-minute Microtox EC_{50} (mg/L Active Ingredients)	1-hour Polyseed IC_{50} (mg/L Active Ingredients)	2-hour Polyseed IC_{50} (mg/L Active Ingredients)	6-hour S. Capricornutum IC_{50} (mg/L Active Ingredients)	24-hour S. Capricornutum IC_{50} (mg/L Active Ingredients)
Pentachlorophenol	1.5	1.2	1.2	1.0	2.2	1.7
Simazine (2-Chloro-4,6-bis (Ethylamino)-S-Triazine)	>450	>450	>450	>450	1.2	0.78
Kathon 886 (5-chloro-2-Methyl -4-Isothiazolin -3-one (11.7%) and 2-Methyl-4-iso-Thiazolin-3-one (3.5%)	0.11	0.050	0.026	0.035	0.021	0.029

3.6.3 Inorganic Chemical Characterization. To assess the quality of the analytical procedures used to characterize the aqueous leachates, a limited quality control program was conducted. This consisted of replicating the analysis of a selected leachate sample at the beginning and at the end of the analytical program, as well as the replicate analysis of the blank leaching medium. The results of this evaluation are shown on Table 8.

The initial and replicate analyses of the selected leachate compare favourably for almost all parameters examined. Minor variation between the two analyses was observed for calcium and potassium in the milligram per litre range while variations in boron, magnesium, silica, strontium and zinc was apparent in the microgram per litre range. This variation was similar to that observed in other characterization studies.

The replicate analyses of the blank leaching media also showed little variation in chemical composition between the two analyses. This suggests that any variation in the characterization in the inorganic constituents is due to variations in the chemistry and leachability of the coal combustion residues rather than changes in the leaching medium.

Minor variations between the chemical characterizations of the two leaching media samples occurred for the analyses of boron, copper, silica, silver and zinc. These are not believed to be significant as these parameters were quantified at a slightly above their detection limit. No parameter was present in concentrations greater than three times its detection limit.

3.7 Summary

Samples of bottom and fly ash from the PCF combustion of representative Canadian coal types and bed and baghouse material from an atmospheric fluidized bed combustion unit were obtained. Split samples were used to prepare aqueous and solvent extracts. These, in addition to two ash-contacted waters from operating pulverized coal fired generating stations, were analyzed for organic halide content, gross inorganic chemical characteristics, and toxicity. Organic halides were quantified using TOC, POX and TOX analyses. The short-term screening procedures used were the Microtox, Polyseed, and algal protocols. Figure 2 summarizes the characterization and analytical program.

TABLE 8 REPLICATE ANALYSES OF LEACHATE AND LEACHING MEDIA

	Leachate (mg/L)		Leaching Media (mg/L)	
Parameter	Initial Analysis	Replicate Analyses	Initial Analysis	Replicate Analyses
Aluminum	< 0.1	< 0.1	0.04	0.04
Barium	0.036	0.036	< 0.005	< 0.005
Beryllium	< 0.005	< 0.005	< 0.005	< 0.005
Boron	0.219	0.238	< 0.004	0.009
Cadmium	0.02	0.03	< 0.01	0.01
Calcium	260	269	0.17	0.17
Chromium	0.19	0.19	< 0.01	< 0.01
Cobalt	< 0.05	< 0.05	0.06	0.06
Copper	< 0.008	< 0.008	0.009	< 0.008
Iron	0.04	0.04	0.02	0.02
Lead	< 0.05	0.05	< 0.05	< 0.05
Magnesium	3.57	3.53	< 0.01	< 0.01
Manganese	< 0.01	< 0.01	< 0.01	< 0.01
Molybdenum	< 0.2	0.2	< 0.2	< 0.2
Nickel	< 0.05	0.06	< 0.05	< 0.05
Phosphorus	< 0.5	< 0.5	< 0.5	< 0.5
Potassium	53	50	1	1
Silica	7.62	7.86	< 0.5	0.07
Silver	< 0.005	< 0.005	0.012	0.011
Sodium	426	426	< 1	< 1
Strontium	0.962	0.966	0.001	< 0.001
Titanium	< 0.005	< 0.005	< 0.005	< 0.005
Vanadium	0.026	0.010	0.010	0.010
Zinc	0.07	0.15	0.05	0.06
Zirconium	< 0.05	< 0.05	< 0.05	< 0.05

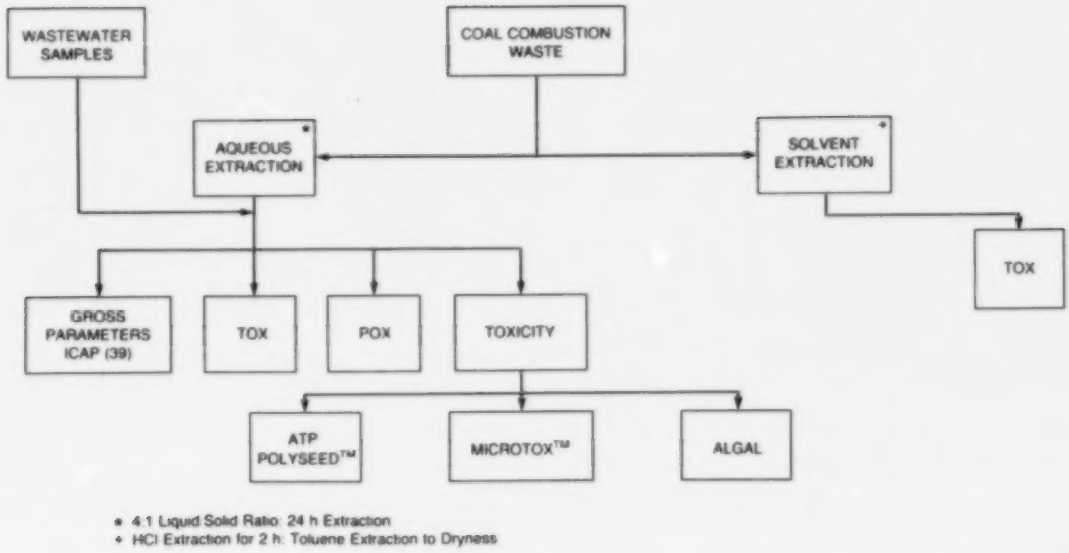

FIGURE 2 ANALYTICAL PROGRAM FLOW CHART

4 RESULTS

4.1 Characterization of Aqueous Extracts

The results of the analyses of the aqueous extracts of combustion residues from the six sites sampled, the NBS standard material, and the leaching media are shown in Table 9. The results of the toxicity assessment (Microtox, Polyseed and algal), the organic screening parameters (TOC, POX and TOX) and the inorganic chemical analyses are included.

The toxicity screening results are expressed as the concentration required to cause 50 percent reduction of activity in the organism as measured by the test procedure. Higher values are associated with lower toxicity. The results ranged widely from 0.5 percent (toxic) to greater than 50 (low or negligible toxicity). The results of the toxicity tests at both incubation times are summarized in Table 10.

Both incubation periods provided similar information. The Microtox assay appeared to show a slightly greater sensitivity with extended incubation but no trend was apparent for the Polyseed organisms. In addition, no major differences were apparent for the two incubation periods used for the algal assay. The results suggest this protocol has good potential for an algal toxicity screening test to be completed within the same day, although algal growth inhibition by the leachates appears in most cases to be significantly greater for the shorter incubation. Presumably, the longer interval provides an opportunity for acclimatization.

For comparison to other parameters, data from the longer incubation periods of the toxicity tests were chosen because the observable difference in net growth between the test conditions and control would be greater. For the Microtox test, the 15-minute incubation period was selected as recommended by Qureshi et al. (89).

The total organic carbon concentrations ranged from less than 1 mg/L to 7.7 mg/L. During the six-week test the detection limit on the TOC analyzer varied between 0.5 and 1 mg/L due to instrument conditions. This accounts for the variation in the number of significant digits reported. The TOC values represent the non-purgeable TOC since volatile components would be lost during the nitrogen sponging used to remove inorganic carbon.

The purgeable organic halide concentrations ranged between 0.0 and 2.7 µg Cl^-/L. For one sample, very high values averaging 963 µg Cl^-/L were observed. It was suspected that these resulted from sulphide interference; samples were yellow-coloured and gave off a strong H_2S odour when acidified. Bean et al. (90) and Bean et al. (91) also

TABLE 9 CHARACTERIZATION OF AQUEOUS EXTRACTS AND ASH SYSTEM WASTEWATERS

	Dalhousie G.S.			Summerside		Lakeview G.S.		Poplar River G.S.		Milner G.S.		Battle River G.S.			Leaching Medium - Deionized Water
Parameter	Sluice Waste	Bottom Ash	Fly Ash	Bed Material	Baghouse Material	Bottom Ash	Fly Ash	Sluice Water	Fly Ash	Bottom Ash	Fly Ash	Bottom Ash	Fly Ash	NBS Fly Ash	
Microtox (%) (15-min EC_{50})	>50	>50	>50*	0.7	0.5	>50	3.5	>50	3.2	17	5.0	>50	5.2	28	>50
Polyseed (%) (2-h IC_{50})	>50	>50	37*	1.0	0.9	>50	6.5	>50	5.0	40.5	8.6	>50	6.5	10.7	>50
Algal (%) (24-h IC_{50})	>50	>50	>50	4.1	4.5	>50	19	>50	39	>50	35.7	>50	2.6	9.45	>50
TOC	1.2	0.5	0.5	1.1	7.7	<1	1.3	5.0	1.0	<1	0.8	2.2	0.4	5.0	1.3
POX ($\mu g\ Cl^-/L$)	0.0	2	2.7	0.0	963**	<2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TOX ($\mu g\ Cl^-/L$)	22.3	11.2	13.1	8.0	31.6	6.7	7.2	10.8	3.2	2.6	8.7	3.0	10.8	12.5	9.2
pH (units)	9.30	10.55	7.00	12.28	12.58	8.95	11.88	9.20	11.92	11.15	11.67	8.35	11.57	9.4	6.7
TDS	2524	262	3203	4689	5146	126	2248	2148	943	220	1033	74	619	-	<2
Alkalinity	70	40	18	1800	2400	24	387	228	497	96	241	50	327	<1	4
Chloride	37	4.2	5.2	12.1	30	<3.0	9.6	21	3.0	<3.0	<3.0	<3.0	<3.0	-	<3.0
Fluoride	1.40	0.20	1.03	0.98	3.9	<0.05	1.37	7.5	3.4	0.50	22	0.10	1.30	-	<0.05
Nitrite	0.04	<0.02	0.17	<0.02	0.04	<0.02	0.03	0.03	<0.02	0.03	<0.02	0.06	<0.02	-	<0.02
Nitrate	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	-	<1
Aluminum	<0.01	1.66	<0.01	<0.01	<0.01	0.15	<0.01	1.34	3.89	2.08	5.51	0.17	15.9	18.1	0.04
Arsenic	1.00	0.42	42	0.007	0.009	0.073	0.104	0.171	0.040	0.124	0.041	0.020	0.007	0.102	<0.005
Barium	0.036	0.016	<0.005	0.130	0.210	0.060	0.737	0.207	1.21	0.180	0.852	0.369	0.457	0.090	<0.005
Beryllium	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	0.0158	<0.0005
Boron	0.219	0.019	1.57	0.116	0.035	0.098	8.57	59.9	34.8	0.388	11.1	0.740	30.0	2.30	<0.004
Cadmium	0.02	0.01	0.03	0.04	0.02	<0.01	0.02	0.01	0.02	<0.01	0.02	<0.01	0.02	0.09	<0.01
Calcium	260	60.1	699	1410	1710	28.9	580	192	281	39.2	291	15.9	188	274	0.17

TABLE 9 CHARACTERIZATION OF AQUEOUS EXTRACTS AND ASH SYSTEM WASTEWATERS (Cont'd)

	Dalhousie G.S.			Summerside		Lakeview G.S.		Poplar River G.S.		Milner G.S.		Battle River G.S.			Leaching Medium -
Parameter	Sluice Waste	Bottom Ash	Fly Ash	Bed Material	Baghouse Material	Bottom Ash	Fly Ash	Sluice Water	Fly Ash	Bottom Ash	Fly Ash	Bottom Ash	Fly Ash	NBS Fly Ash	Deionized Water
Chromium	0.19	0.02	0.03	0.04	0.03	<0.01	0.58	0.29	0.43	0.02	1.18	0.02	0.30	0.02	<0.01
Cobalt	<0.05	<0.05	0.08	0.10	0.07	<0.05	<0.05	<0.05	0.06	<0.05	0.05	0.10	0.06	0.14	0.06
Copper	<0.008	<0.008	0.013	0.020	0.021	<0.008	<0.008	0.010	<0.008	<0.008	<0.008	<0.008	<0.008	0.835	0.009
Iron	0.04	0.05	0.12	0.18	0.17	0.02	0.09	0.04	0.08	0.03	0.10	0.04	0.09	0.23	0.02
Lead	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Magnesium	3.57	0.23	10.1	<0.01	<0.01	3.85	0.04	26.8	0.02	0.23	0.03	2.12	0.03	17.5	<0.01
Manganese	<0.01	<0.01	1.58	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.61	<0.01
Molybdenum	<0.2	<0.2	6.4	<0.2	<0.2	<0.2	2.8	1.3	0.5	<0.2	1.2	<0.2	0.3	<0.2	<0.2
Nickel	<0.05	<0.05	0.14	0.13	0.15	<0.05	0.08	<0.05	0.08	<0.05	0.05	<0.05	<0.05	0.51	<0.05
Phosphorus	<0.5	<0.5	4.4	0.8	0.8	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Potassium	53	<1	94	10	<1	<1	45	36	11	5	25	3	2	56	<1
Silver	<0.005	<.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.012	<0.005	<0.005	0.012
Sodium	426	12	44	3	<1	2	56	339	15	41	23	18	38	20	<1
Strontium	0.962	0.139	1.49	6.17	3.49	0.214	9.86	5.32	10.1	0.524	3.18	0.200	5.47	2.95	<0.001
Titanium	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.014	<0.005
Silica	7.62	4.44	34.5	0.16	0.26	1.97	4.72	1.03	4.01	14.5	3.88	4.24	0.75	16.9	<0.05
Vanadium	0.026	0.033	0.202	0.027	0.012	0.014	0.162	0.134	0.044	0.113	0.326	0.028	0.031	0.031	0.010
Zinc	0.07	<0.05	0.11	0.14	0.15	<0.05	0.08	<0.05	0.07	<0.05	0.07	<0.05	0.05	1.15	0.05
Zirconium	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

NOTE: Results expressed as mean of triplicate analyses for fly ashes; single analyses for all others. Units are mg/L unless otherwise indicated.

* Replicate analyses only.
** Suspected sulphide interference on POX analyses, see text.

TABLE 10 COMPARISON OF TOXICITY VALUES AT TWO INCUBATION PERIODS FOR EACH TEST PROCEDURE

	Microtox EC_{50}[a]		Polyseed IC_{50}[b]		Selenastrum capricornutum IC_{50}[c]	
Sample	5 min	15 min	1 h	2 h	6 h	24 h
Dalhousie G.S.						
Sluice water	>50	>50	>50	>50	>50	>50
Bottom ash	>50	>50	>50	>50	>50	>50
Fly ash	>50	>50	>50	42%	>50	>50
Fly ash	>50	>50	>50	50	>50	>50
Fly ash	>50	48%	20%	32%	>50	>50
Summerside						
Bed material	0.75%	0.85%	1.2%	1.1%	1.8%	4.1%
Bed material	0.65%	0.60%	0.74%	0.84%	1.4%	2.5%
Bed material	0.70%	0.68%	0.58%	0.92%	<3.9%	5.7%
Baghouse material	0.81%	0.65%	1.7%	1.5%	1.3%	5.0%
Baghouse material	0.53%	0.38%	0.49%	0.63%	1.3%	4.3%
Baghouse material	0.60%	0.56%	0.80%	0.64%	1.4%	4.2%
Lakeview G.S.						
Bottom ash	>50	>50	>50	>50	>50	>50
Fly ash	5.9%	4.5%	7.0%	6.2%	<9.1%	18%
Fly ash	3.6%	30%	8.2%	8.3%	12%	22%
Fly ash	3.2%	2.9%	4.2%	5.1%	<5.6%	17%
Poplar River G.S.						
Sluice water	>50	>50	>50	>50	>50	>50
Fly ash	3.4%	3.1%	3.2%	4.7%	13%	21%
Fly ash	2.2%	4.0%	6.8%	6.3%	>17%	83%
Fly ash	2.8%	2.4%	4.6%	4.1%	5.5%	13%
Milner G.S.						
Bottom ash	22%	17%	28%	41%	>50	>50
Fly ash	6.5%	5.4%	11%	10%	20%	30%
Fly ash	5.5%	4.9%	16%	7.6%	16%	56%
Fly ash	5.4%	4.6%	8.0%	8.3%	8.0%	21%
Battle River G.S.						
Bottom ash	>50	>50	>50	>50	>50	>50
Fly ash	7.5%	6.5%	7.9%	6.6%	0.42%	0.88%
Fly ash	5.6%	4.5%	<3.6%	4.4%	<5.7%	3.5%
Fly ash	6.3%	4.7%	4.5%	8.5%	2.5%	3.4%
NBS Fly ash	39%	28%	8.9%	11%	11%	9.5%
Leaching Medium	>50	>50	>50	>50	>50	>50

[a] EC_{50} is the concentration of sample expressed as a percentage of the undiluted sample which caused a 50% reduction of light emitted by the Microtox bacteria after 5 min. (5-min ED_{50}) and 15 min (15-min ED_{50}).

[b] IC_{50} is the concentration of sample expressed as a percentage of undiluted sample which caused a 50% inhibition of growth as indicated by ATP levels after 1 h (1-h IC_{50}) of incubation.

[c] IC_{50} is the concentration of sample expressed as a percentage of undiluted sample which caused a 50% inhibition of growth as indicated by ATP levels after 6 h (6-h IC_{50}) and 24 h (24-h IC_{50}) of incubation.

identified analytical problems associated with this method due to the presence of sulphur compounds. After acidification, the POX was 0.0 µg Cl^-/L, and has been considered insignificant. Total organic halide concentrations ranging between 2.6 and 31.6 µg Cl^-/L were found in all samples.

The aqueous extracts tended to be alkaline, in the pH range of 7.0 to 12.58. The NBS fly ash extract had a pH of 4.4. The total dissolved solids (TDS) concentrations ranged widely between 126 and 5146 mg/L.

It is difficult to generalize the trends in concentrations of the other inorganic parameters. Some were nondetectable in all samples, such as beryllium, silver and titanium, while others varied greatly between samples, such as aluminum, arsenic, calcium and sodium. Variation in the chemical characteristics is probably due to variation in the chemical composition and leachability of the coal combustion residues.

Table 11 shows the mean, standard deviation and coefficient of variation for the Microtox, Polyseed and algal toxicity screening tests. These data are based on triplicate analyses with one exception where replicate values are used. The coefficient of variation is a measure of relationship between the standard deviation of a distribution to the mean of the distribution. This permits distributions about differing magnitudes of means to be compared. The coefficients of variation were 0.2, 0.3 and 0.4 for the Microtox, Polyseed and algal tests, respectively.

Table 12 shows the mean, standard deviation and coefficient of variation for the TOC, POX and POX analyses. These are based on six analyses of the same sample. The coefficients of variation were 0.6, 0.3 and 0.4 for the TOC, POX, and TOX, respectively.

4.2 Total Extractable Organohalides

As previously mentioned, split samples of the combustion residues were subjected to solvent extraction with the extracts subsequently analyzed for TOX. These results are shown on Table 13. The second column shows the total extractable organic halides expressed in µg Cl^-/g of sample. For simplicity, true zero readings (0.00 µg in 5 nL) and readings less than the detection limit (0.01 or 0.02 µg in 5 nL) have been expressed as less than the detection limit.

A method blank averaging 26 mg Cl^-/mL (2.5 µg Cl^-/g sample assuming a 10 g sample) was observed. This was greater than 11 of the sample determinations and cannot be explained. For this reason the third column of numbers was calculated. These represent the blank corrected total extractable organohalides expressed in µg Cl^-/g of

TABLE 11 VARIATIONS IN REPLICATE MICROTOX™, POLYSEED™ AND ALGAL TOXICITY TESTING

Sample	Microtox			Polyseed			Algal		
	Mean	Standard Deviation	Coeff. of Variation	Mean	Standard Deviation	Coeff. of Variation	Mean	Standard Deviation	Coeff. of Variation
Dalhousie G.S.									
Fly ash	>50*	>50*	0	37*	7.1*	0.2	>50	>50	0.0
Summerside									
bed material	0.7	0.1	0.2	1.0	0.1	0.1	4.1	1.6	0.4
baghouse material	0.5	0.1	0.2	0.9	0.5	0.6	4.5	0.4	0.1
Lakeview G.S.									
Fly ash	3.5	0.9	0.3	6.5	1.6	0.3	19	2.6	0.1
Poplar River G.S.									
Fly ash	3.2	0.8	0.3	5.0	1.1	0.2	39	38	1.0
Milner G.S.									
Fly ash	5.0	0.4	0.1	8.6	1.2	0.1	35.7	18.2	0.5
Battle River G.S.									
Fly ash	5.2	1.1	0.2	6.5	2.1	0.3	2.6	1.5	0.6
Average			0.2			0.3			0.4

* replicate analyses only

TABLE 12 VARIATIONS IN REPLICATE ANALYSES FOR TOC, POX AND TOX

Sample	TOC			POX			TOX		
	Mean	Standard Deviation	Coeff. of Variation	Mean	Standard Deviation	Coeff. of Variation	Mean	Standard Deviation	Coeff. of Variation
Dalhousie G.S.									
Fly ash	0.5*	0.5*	1.0	2.7*	3.1*	1.1	13.1*	1.8*	0.1
Summerside									
bed material	1.1	0.2	0.2	0.0	0.0	0.0	8.0	6.0	0.8
baghouse material	7.7	2.9	0.4	963	936	1.0	31.6	19.1	0.6
Lakeview G.S.									
Fly ash	1.3	0.8	0.6	0.0	0.0	0.0	7.2	3.6	0.5
Poplar River G.S.									
Fly ash	1.0	0.2	0.2	0.0	0.0	0.0	3.2	0.8	0.3
Milner G.S.									
Fly ash	0.8	1.0	1.3	0.0	0.0	0.0	8.7	2.8	0.3
Battle River G.S.									
Fly ash	0.4	0.3	0.6	0.0	0.0	0.0	10.8	5.1	0.5
Average			0.6			0.3			0.4

* replicate analyses only

TABLE 13 TOTAL EXTRACTABLE ORGANOHALIDES

Sample	Total Extractable Organic Halides (μg Cl^-/g sample)	Blank Corrected Total Extractable Organic Halides (μg Cl^-/g sample)
NBS Fly Ash 1633a	6.2	4
Dalhousie G.S.-Bottom Ash	>0.5	>1
Dalhousie G.S.-Fly Ash*	>0.5	>1
Summerside-bed material	1.0	>1
Summerside-baghouse material	>0.5	>1
Lakeview G.S.-bottom ash*	0.95	>1
Lakeview G.S.-fly ash	3.1	>1
Milner G.S.-bottom ash	0.7	>1
Milner G.S.-fly ash	7.7	5
Battle River G.S.-bottom ash	3.6	>1
Blank	2.5**	-

* indicates average of replicate analyses.
** calculated assuming 10 g of sample.

sample. These values are generally less than the calculated detection limit of 1; however, two values of 4 and 5 μg Cl^-/g were observed.

4.3 TOX Analyses of Known Organohalides

In order to verify the analytical procedure for the TOX analyses, three known chlorinated organic compounds, selected to represent a wide range of chemical structures, were analyzed. They were pentachlorophenol, 2-chloro-4,6-bis(ethylamine)-5-triazine, also known as simazine and 5-chloro-2-methyl-4-isothiazolin-3-one (Kathon 866). This is shown on Table 14. These compounds were also selected because Microtox values for their toxicity have been reported in the literature. The recoveries ranged between 102 and 169 percent. This was not unexpected since these probably contained chlorinated compounds other than the active ingredients which would be quantified by the TOX determination.

TABLE 14 TOX ANALYSES OF KNOWN TOXIC COMPOUNDS

Compound and Description	Expected TOX (mg Cl^-/g)	Measured TOX (mg Cl^-/g)	Recovery Percent
Pentachlorophenol - technical grade - minimum 95% total chlorinated phenols, minimum 86% as pentachlorophenol	572 (86% PCP)	588	103
Algi-Gon - 0.1% 2-chlor-4, 6-bis (ethylamine) (Simazine)*	176 (1% Simazine)	298	169
Kathon 886 - 1.01% 5-chloro-2-methyl-4-isothiazolin-3-one*	2371 (1.01%)	3998	169

* These may have contained chlorinated organic compounds other than the active ingredients which would be quantified by the TOX determination.

5 DISCUSSION

The following discussion of the analytical results is focused on the samples examined during this investigation, and on the applications or limitations of the procedures used.

5.1 TOC, POX and TOX Analyses

Total organic carbon concentrations ranged between less than 1 to 7.7 mg/L in the aqueous extracts. With the exception of wastes from Battle River G.S., extracts from bottom ash contained lower or the same concentration as those from the fly ash from the same site. The TOC concentration in aqueous extracts from the NBS standard exceeded all other pulverized coal fired combustion ash aqueous extracts. Extracts from the atmospheric fluidized bed baghouse material sample contained TOC concentrations several times greater than those from PCF fly ashes.

In the case of the Dalhousie G.S., the TOC concentration in the ash sluice water sample from the plant was about two-and-a-half times greater than that of the aqueous extracts from the ash produced in the laboratory. This may be due to non-ash related wastes in the sluice water system or to the chemical treatment used in that system. A similar case exists for Poplar River G.S., where the ash sluice water contains five times the TOC quantified in the laboratory-produced aqueous extracts. This may be due to routing non-ash station wastewaters to the ash lagoons in this waste stream.

Purgeable organohalides were quantified in the aqueous extracts from bottom and fly ash from Dalhousie G.S. The concentrations only slightly exceeded the detection limit for this analysis. It was not unexpected that no or very low POX concentrations were found. During combustion, sampling and leaching, there are many opportunities for purgeable or volatile organics to be lost. With this perspective, further POX analyses do not appear to be worthwhile for this type of combustion residue sample, leached in this manner.

Measurable TOX concentrations were found in all samples. These ranged from 2.6 to 31.6 $\mu g\ Cl^-/L$ in the aqueous extracts. Although the TOX concentrations in the leaching medium are significant, the concentrations in most of the samples were even lower, making the interpretation of these results difficult. Only two samples had concentrations greater than twice the leaching medium. These were the sluice water from Dalhousie G.S. (22.3 $\mu g\ Cl^-/L$) and the extracts from the Summerside AFBC unit baghouse material (31.6 $\mu g\ Cl^-/L$).

During the TOX analyses, three different qualities of deionized water were evaluated as alternative leaching media. The TOX concentrations ranged from 16.7 µg Cl^-/L for Anachemica Accussolve water, 6.4 µg Cl^-/L for Milli-Q water and as low as 0.3 µg Cl^-/L for Millipore Organex ultra pure water. The water most free of TOX (Organex) gave concentrations of 0.3, 1.5 and 2.8 µg Cl^-/L over a one-month testing period. This suggests that it may be possible to further reduce the working detection limit of the analysis by the use of a higher cost, lower TOX-content leaching medium. This would need to be demonstrated with further testing under more rigorous laboratory control.

TOX concentrations were also found to be correlated ($r = 0.740$) to the chloride concentration in the aqueous extracts. Only inorganic chlorides were quantified by the ion chromotographic method used during this study. This procedure cannot be used to determine organochlorides. However, it might be expected that coals yielding ashes with high chloride concentrations might also favour the formation of organochloride species.

In terms of its use as a screening tool, TOC appears to be capable of quantifying organic carbon in aqueous extracts in the greater than 1 mg/L range. Concentrations in the aqueous extracts ranged from 1 to 7.7 mg/L, the same order of magnitude as reported in the literature (11). Based on very limited data, TOC might be used as a screening procedure for TOX since a high correlation between the two results was found.

Detection limits appear to be adequate for use of TOX as a screening tool in leachate analysis but a potential for interference by high levels of inorganics exists. The quality control work showed that high Cl^-, Br^- and $SO_4^=$ can give false positive organohalide values. Hydrogen sulphide poses a potential interference in POX determination of leachates.

The research of Riggin et al (5) indicated that, for most water samples, more organohalides are recovered by the GAC absorption/pyrolysis/micro-coulimetric technique than by the extraction GC procedures of U.S. Environmental Protection Agency Methods 625 and 612. Better recovery of polar compounds is to be expected and there is less chance of losing volatile halogens than during the concentration steps of a solvent extraction procedure.

One purpose of this study was to examine TOX as a screening tool to detect potential hazards in leached solid waste, but the TOX levels found were less than that in chlorinated tap water. For example, in the chlorinated municipal tap water in

Mississauga, Ontario, the concentration of TOX has been found to be 69 µg/L Cl^-. None of the leachate samples tested exceeded this amount.

Table 15 presents the concentrations of blank corrected total extractable organic halides, aqueous extractable organic halides in ash contacted wastewaters. Blank corrected total extractable organic halide concentration greater than the detection limit were found only in two samples: the NBS standard fly ash and fly ash from Milner G.S.

The laboratory-produced aqueous extracted organic halides and organic halides in ash-contacted wastewater from generating stations can be compared for bottom ash at Dalhousie G.S. and fly ash at Poplar River G.S. In both cases the concentrations in the ash-contacted water were two and three times greater than in the laboratory-produced extracts. Since both these ash systems operate in a recirculating mode, it is possible that the aqueous extractable organohalides are increased in concentration by the recirculation of the sluice water. The greater concentrations may also be due to extended, more rigorous contact between the ash and sluice water resulting in a higher rate of extraction than that used in the laboratory.

The absolute amount the TOX analyzer can safely detect is 0.02 µg Cl^-. This detection limit using an unmodified Dohrmann DX-20 system, is inadequate for comparing a water leachate to an organic solvent extract. In the first case, a 25-g sample is leached with 500 mL of water and a 100-mL aliquot is examined for TOX, providing a detection limit of leachable TOX of 0.004 µg Cl^-/g. The total extractable organic halide procedure requires a solvent injection and it was found that a maximum of 10 µg of solvent (either toluene or iso-octane) could safely and reproducibly be introduced into an unmodified pyrolysis unit. Even using a 10-g sample and risking loss of the more volatile organohalides by concentrating the sample to 1 mL, the best possible detection limit for solvent extraction would be 0.2 µg Cl^-/g sample. There is a 50-fold difference between the amount detectable by water leaching and solvent extraction.

The TEOX procedure used in this study approximated that of Coburn et al. (28) and the minimum detectable amount of organic halides was found to be 50-fold poorer than that found by Coburn et al. even without blank correction. The blank values found in this study tended to agree with Riggins (5) at the 2-3 µg level whereas Coburn et al. apparently had an average background of 0.1 µg/g.

In this study, only two samples had extractable organohalides that exceeded the true detection limit of the method; that is, twice the background reading.

TABLE 15 COMPARISON OF TOTAL EXTRACTABLE AND AQUEOUS EXTRACTABLE ORGANIC HALIDES

Sample	Total Extractable Organic Halides ($\mu g\ Cl^-/g$ sample)	Blank Corrected Total Extractable Organic Halides ($\mu g\ Cl^-/g$ sample)	Aqueous Extractable Organic Halides ($\mu g\ Cl^-/mL$)	Organic Halides in Ash-Contacted Wastewaters ($\mu g\ Cl^-/mL$)
NBS Fly Ash 1633a	6.2	4	0.0092	-
Dalhousie G.S.-Bottom Ash	<0.5	<1	0.0112	0.0223
Dalhousie G.S.-Fly Ash*	<0.5	<1	0.0131	-
Summerside-bed material	1.0	<1	0.008	-
Summerside-baghouse material	<0.5	<1	0.0316	-
Lakeview G.S.-bottom ash*	0.95	<1	0.006	-
Lakeview G.S.-fly ash	3.1	<1	0.0072	-
Poplar River G.S.-fly ash	0.6	<1	0.0032	0.0108
Milner G.S.-bottom ash	0.7	<1	0.0026	-
Milner G.S.-fly ash	7.7	5	0.0087	-
Battle River G.S.-bottom ash	3.6	<1	0.003	-
Battle River G.S.-fly ash	1.7	<1	0.0108	-
Blank	2.5**	-	0.0125	-

* indicates average of replicate analyses.
** calculated assuming 10 g of sample.

5.2 Toxicity Screening Procedures

Three screening procedures were used to assess the toxicity of the laboratory-produced aqueous extracts and ash-contacted wastewaters. Aqueous extracts from all bottom ash samples were shown to have negligible toxicity as determined by the three procedures. Likewise the ash-contacted sluice waters from Dalhousie G.S. and Poplar River G.S. and the leaching medium also appeared to have negligible toxicity based on the results of this characterization.

In terms of the Microtox procedure, aqueous extracts from two AFBC residues from Summerside showed the greatest toxicity with EC_{50} values in the range of 0.5 to 0.7%. Fly ashes from Lakeview G.S. and Poplar River G.S. were relatively less toxic with values of 3.5 and 3.2%, respectively. Fly ashes from Milner G.S. and Battle River G.S. were even less toxic with values of 5.0 and 5.2%. These can be compared to the EC_{50} from the Microtox procedure for the NBS fly ash of 28% which indicates relatively less toxicity.

Similar results were obtained using the Polyseed protocol. The IC_{50} values indicate the most toxic aqueous extracts were from the AFBC residues, followed by fly ash from Poplar River G.S., Lakeview G.S., Battle River G.S. and Milner G.S.. Again the NBS fly ash aqueous extract was shown to be the less toxic than all fly ashes except that from Dalhousie G.S.

Results from the algal screening procedure gave slightly different results. Aqueous extracts from Battle River G.S. fly ash were the most toxic followed by those from the AFBC wastes from Summerside, Lakeview G.S., Milner G.S. and Poplar River G.S. Aqueous extracts from the NBS fly ash gave toxicities which fell into the middle of the range observed during the testing.

To investigate the origin of the observed toxicities as measured by the three screening procedures, correlations were determined. The correlation coefficient was calculated for each pair of parameters, e.g., observed toxicity as determined by the Microtox method, and pH. The value of the coefficient can range from -1, indicating perfect negative correlation, to +1 indicating perfect positive correlation. A value of 0 indicates no correlation. Table 16 summarizes these calculations in a correlation matrix. Correlations between a parameter and the three screening procedures that were insignificant are not shown. Numerical values for the correlations in the range of -1.0 to -0.7 and +0.7 to +1.0 are shown.

The toxicity of the aqueous extracts as measured by the Microtox, Polyseed and algal procedures could not be correlated to the concentration of TOC or TOX in the

TABLE 16 CORRELATION MATRIX

Parameter	Microtox	Polyseed	Algal
TOC	0	0	0
TOX	0	0	0
Microtox	1	0.989	0
Polyseed	0.989	1	0
Algal	0	0	1
pH	-0.907	-0.738	0
Copper	0.786	0	0
Magnesium	0.849	0	0
Zinc	0.790	0	0
Barium	0	0	0.895
Chromium	0	0	0.750
Silica	0.950	0.808	0

NOTE: Correlation coefficient ranges between -1 indicating perfect negative correlation (increased toxicity with increasing concentration) and +1 indicating perfect positive correlation (decreased toxicity with increasing concentration). Only correlation coefficients in the range -1 to 0.7 and 0.7 to 1.0 are shown. "0" indicates no correlation (r = -0.7 to +0.7)

sample. This is not unexpected since concentrations of TOC were only slightly above the detection limit in most of the samples. Likewise, TOX concentrations above those of the blank leaching medium were quantified in only a limited number of samples. The results of the Microtox protocol were highly correlated with the Polyseed results (r=0.989). This relationship is surprising since the Microtox organism was chosen to be very sensitive to toxicants, whereas the Polyseed consists of a mixed population intended for use as a BOD seed. The mixed population might be expected to be more resistant to the insult of a toxicant.

In general, the toxicity indicated by a test procedure is a function of the test organism and the test environment. As such, not all toxic effects will be manifested by all organisms in all environments. The correlation or lack of correlation among the results of the three toxicity screening procedures indicates the degree of sharing the toxic effects between the various organisms and environmental conditions.

The usefulness of a multiple procedure toxicity screening approach is apparent when the correlation matrix is considered in terms of the inorganic parameters. No one parameter was correlated with the toxicity as determined by all three toxicity screening tests. Toxicity as measured by the Microtox protocol only was negatively correlated with pH and positively correlated with copper, magnesium and zinc. Thus aqueous extracts

with high pH were more toxic while greater concentrations of copper, magnesium and zinc were associated with lower toxicity.

In the case of the algal procedure, toxicity was negatively correlated with pH if the results of the testing of the NBS fly ash are excluded, and positively correlated with barium and chromium. The results indicate that increased toxicity is related to increased pH values and increased concentrations of barium and chromium would appear to reduce the toxicity of the aqueous extracts as measured using this procedure.

Silica was positively correlated in both the Microtox and Polyseed methods. This indicates that increased silica concentrations were related to lower toxicity.

The strong correlations between the Microtox toxicity and pH, and between the Polyseed toxicity and pH, were expected. Both the technical literature and procedure manual for Microtox identify pH as a cause of toxicity. The lack of correlation between pH and toxicity as determined by the algal method was, however, unexpected. In fact, during the toxicity characterizations, pH was used as the basis for selecting the appropriate dilution of aqueous extracts prior to determining their toxicity. This suggests that the algal population is less sensitive to alkaline aqueous conditions and is consistent with the results of Brezonik et al. (48). They observed only a relatively small decrease of ATP in cultures of S. capricornutum on exposure to an environment of pH 10. With the exception of pH, no other strong negative correlation occurred between a parameter and toxicity using the three procedures.

Several parameters were positively correlated with, toxicity, indicating decreasing toxicity with increasing concentration. Although these effects may be related to a number of interactions, this cannot be verified by examination of the data. For example, at the concentrations determined in the aqueous extracts, copper, magnesium, and zinc may be acting as micronutrients for the Microtox organism, providing a growth stimulation effect. For the algal toxicity test procedure, barium may be co-precipitating with other metals, reducing the overall toxicity of the aqueous extract.

6 SUMMARY AND CONCLUSIONS

6.1 Summary

This study was conducted to develop and examine a battery of test protocols for the characterization of coal combustion residues. Little information is available on the characteristics of these residues in terms of their organohalide content or the effects of these contaminants and their combination with inorganic characteristics on bioassay (toxicity) test species. Because bioassays can simultaneously assess the effects of numerous toxicants, they are useful screening procedures and can be used to develop comparative toxicity data between combustion wastes from different coals. Bioassay data, together with toxicant data and relevant inorganic chemical information can be used to guide the formulation of waste disposal and wastewater discharge criteria to reduce adverse effects on the environment.

This study characterized aqueous and to a limited extent solvent extracts of coal combustion wastes from representative coals across Canada burned in pulverized coal and atmospheric fluidized bed combustors. Emphasis was placed on the characterization of aqueous extracts, which would be most representative of leachates likely to be released into the aquatic environment.

The characterization program consisted of screening for organohalides and short-term toxicity tests. The organic components were quantified using total organic carbon, total organic halide, and purgeable organic halide analyses. The toxicity screening procedures were the Microtox, an ATP/Polyseed and an algal method. All extraction and characterization methods were selected based on a critical review of the literature.

In terms of the inorganic constituents, the aqueous extracts were similar to those reported by many other researchers. The chemical constituents were primarily a function of the coal from which the residues were derived with elemental concentrations governed by the chemistry and physical properties of the wastes. Since the aqueous extracts were filtered prior to characterization, the constitutents should be considered to be in a dissolved form. However, it must be noted that one mechanism to transport toxicants through the ecosystem is through the movement of suspended solids in the bottom sediments of streams or lakes. For example, the solubility of organic compounds or minerals in acidic media such as the stomach fluids of fish may result in toxicity even when dissolved concentrations in the surrounding water are below harmful concentrations.

Other toxic effects may occur through inhalation or cellular ingestion mechanisms, but suspended solids were not considered during this study. A more comprehensive assessment of the risk of toxicity should also address these mechanisms, and the toxicity of the particles before and after aqueous leaching.

6.2 Organic Halides

Total organic carbon (TOC) analysis appears to be useful as a coarse screening procedure for organic compounds in coal combustion residues. Measureable concentrations were found for AFBC residues and one ash-contacted sluice water. This method was limited by its relatively high detection limit of 1 mg/L. TOC concentration in the aqueous extracts were of the same order of magnitude as those reported in the literature.

Purgeable organic halides were not found in any aqueous extracts at levels greater than twice the detection limit. This is not surprising since there are many opportunities for volatile compounds to escape from the residue during combustion and ash handling at the generating station and during aqueous extraction. Losses during the extraction step might be reduced by selecting a protocol with this in mind. However, this would limit the type of comparisons which could be made with the ash leaching studies. In one case, very high POX concentrations were found. Based on the results of the quality control program it is believed that this was due to a strong positive interference from sulphides in the sample. This interference has been suggested by others, but no remedy has been developed for the problem. Elimination of the interference reduced the POX concentration to nondetectable levels. This situation underscores the usefulness of a detailed chemical characterization and quality control in the interpretation of analytical data.

Measureable total organic halide concentrations were found in all samples. However, since many sample TOX concentrations were less than that of the leaching medium, interpretation of this data is difficult. In only one case, aqueous extracts from AFBC baghouse material, were the concentrations greater than twice the concentration in the blank. Also, the ash-contacted wastewater from one site had a TOX concentration greater than twice the detection limit. In comparison, chlorinated tap water from Mississauga, Ontario, contained twice and three times the TOX concentration of these samples, respectively.

Total extractable organic halides were also determined in solvent extracts from the coal combustion residues. Again, high blank or background concentrations make

the interpretation of these results difficult. Two samples, the NBS standard and the Milner G.S. fly ash, had TEOX concentrations five times greater than the detection limit. The fuel at Milner G.S. is a blend of coal rejects with fly ash captured using a baghouse. Further study is required to confirm that the high concentration is related to this fuel or this emission control technology (note that the AFBC station also uses baghouse stack emission control technology).

An original intent of this study was to compare concentrations of TOX and TEOX in the combustion residues in order to estimate the amount of organic halides extracted using water as the medium rather than the more widely used solvent. This was not possible. A 50-fold difference in detection limit between concentrations quantified in aqueous and solvent extracts was found. The blank values found in this study agree favourably with the work of Riggins et al. (5) while the minimum detection limit and background values were 20 to 30 and 50 times greater, respectively, than those reported by Coburn et al. (28).

For the organohalide analyses, the coefficients of variation were 0.6 for the TOC, 0.3 for the POX and 0.4 for the TOX. Recoveries of known chloroorganic compounds and the coefficients of variation indicate that these are practicable characterization protocols. A significant conclusion is that the aqueous extracts are relatively free of organic halides. The exception appears to be the baghouse material from the AFB combustor at Summerside. Further investigation into this waste is warranted.

Initially all samples for organic halide characterization were stored in amber headspace-free bottles with foil-lined caps. This is standard procedure for sample containment. It was found that the foil cap lining frequently corroded and a white precipate formed in the samples. It is believed that this was due to the high pH of the samples. To eliminate this problem, Teflon-lined caps were required for the sample containers. It is suggested that this type of sample container cap be used in the future.

6.3 Toxicity Screening Procedures

Based on a literature review, a battery of three rapid toxicity screening procedures were selected to characterize the aqueous extracts. The Microtox procedure has been widely applied and uses an organism which was selected to be very sensitive to toxicants. Growth inhibition of a mixed microbial population, commercially available as a freeze-dried product known as Polyseed was measured by the ATP (adenosine triphosphate) assay. The response of a primary producer was determined using a protocol

with Selenastrum capricornutum Printz, a species of green fresh water algae commonly used in algal assays.

Aqueous extracts could be grouped by their toxicity as measured by the three protocols. Aqueous extracts from PCF bottom ashes and ash-contacted wastewater were found to be nontoxic or had very low toxicity. As the screening protocol would identify these as having a low probability of causing an adverse impact on the aquatic environment, further characterization of this type of combustion residues would appear to have low priority. However, it should be noted that actual ash-contacted wastewaters from generating stations had slightly higher toxicities than those generated by the laboratory leaching procedure.

Measurable toxicity was found for aqueous extracts from all fly ashes with the exception of the residue from Dalhousie G.S. The highest toxicities were associated with the AFBC residues from Summerside. In terms of microbial groups, the algae were in all but one case less susceptible to toxicants than the other test organisms.

With respect to the test procedures, the Microtox and Polyseed appear to be practicable. The sensitivity of the procedure using the Polyseed culture was surprisingly good. For the algal method, further refinement and optimization is possible. A limitation of this test is the need to maintain a population of the test organism between toxicity screenings and to be familiar with aseptic laboratory techniques.

Toxicity could not be correlated to the presence of organohalides primarily because of their very low or nondetectable concentrations in the aqueous extracts. There was an unexpectedly high correlation betwween toxicity as determined by the Microtox and the Polyseed methods. It was anticipated the former would have been significantly more sensitive since the test organism was selected for its sensitivity. In contrast, the Polyseed organisms were originally selected for use in BOD testing. Furthermore, Polyseed is a mixed culture and as such would have been expected to be more resilient to the insult of a toxicant.

Increased toxicity was highly correlated to increased pH for both the Microtox and Polyseed methods. The cause of this correlation cannot be determined from the results of this study. Further studies should be conducted in which the pH's of aqueous extracts are neutralized. These would then need to be reexamined for toxicity. Methods of pH adjustment might include those commonly used in wastewater treatment.

Reduced toxicity was associated with increased concentrations of several elements. This mechanism cannot be identified based on the results of this study but it can be speculated that these elements may be acting as micronutrients or they could be

reducing the toxicity of other elements by interacting with the water chemistry. This can only be ascertained by detailed experimental designed for this purpose.

In this study, only acute toxic effects were monitored. It would also be desirable to perform additional tests to measure chronic toxicities. Furthermore, a limitation of all short-term bioassays is that the ability of the test organisms to acclimatize to a toxic insult is not evaluated. Because the short-term tests may tend to over-estimate the toxic threat to the environment, it would be desirable to perform growth inhibition assays with extended exposure times.

In summary, the characterization of coal combustion residues using a screening procedure involving organic halide analyses, rapid toxicity testing and inorganic chemical characterization appears to be promising. These methods were found to be practicable; however, several limitations and areas for further refinements of the techniques were identified. The organic halide concentration of the aqueous extracts from the residue samples characterized during this study were very low. Toxicity was not correlated with the organohalides but rather with the inorganic chemical characteristics of the aqueous extracts.

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APPENDIX

TURNER DESIGNS MODEL 20 PHOTOMETER AND BECKMAN MICROTOX™ MODEL 2055 TOXICITY ANALYZER: PERFORMANCE COMPARISON

The following is an excerpt from an unpublished report by J.E. Cairns commissioned by Mono Research Laboratories (Brampton, Ontario) and is presented with their permission.

APPENDIX -

Turner Designs Model 20 Photometer and Beckman Microtox™ Model 2055 Toxicity Analyzer Performance Comparison

1. Usefulness for ATP Assays

A key requirement for ATP analyses is adequate sensitivity. For example, samples for analysis from biocide control applications, environmental biomass surveys, microbial contamination testing, and analytical biochemistry testing very often contain an ATP content of only several picograms (10^{-12} gms) of ATP per mL. The following describes an experiment to compare the sensitivity of the Beckman and Turner Designs instruments for ATP analysis.

One hundred microlitres of an ATP standard solution were mixed in a Beckman Microtox cuvette with one hundred microlitres of Turner Designs Firefly luciferase reagent. Immediately after mixing the reagents, the cuvette was placed in a Beckman Microtox Analyzer, set at maximum sensitivity and a reading was taken from the instrument over a ten second period. Immediately following this reading, the same cuvette was placed in the Turner Designs Photometer, set according to the manufacturer's instructions for routine testing, and after a five second delay, the light was measured in the integration mode for ten seconds. This procedure was repeated using several dilutions of the ATP standard solution and a standard curve for each instrument was produced.

The results of the experiment, shown on Figure 1, demonstrate the excellent sensitivity of the Turner Designs instrument which is orders of magnitude greater than the Beckman Analyzer. Because it would be difficult to measure ATP solutions even in the nanogram (10^{-9} gm)/mL range with the Beckman Analyzer, this instrument would not be useful for the majority of applications for the ATP assay.

2. Usefulness for the Microtox Assay

The Beckman Microtox Analyzer provides a controlled temperature (usually 15°C) for the Microtox bacterial reagent and measures the decrease in light produced by the reagent when it is exposed to a toxicant. The temperature control is provided in an incubator block while the reagent is incubated with the toxicant and is also provided in the photochamber while the light from the reagent is being measured. The following sections demonstrate that the Turner Designs Photometer in combination with controlled temperature recirculators can achieve the same performance.

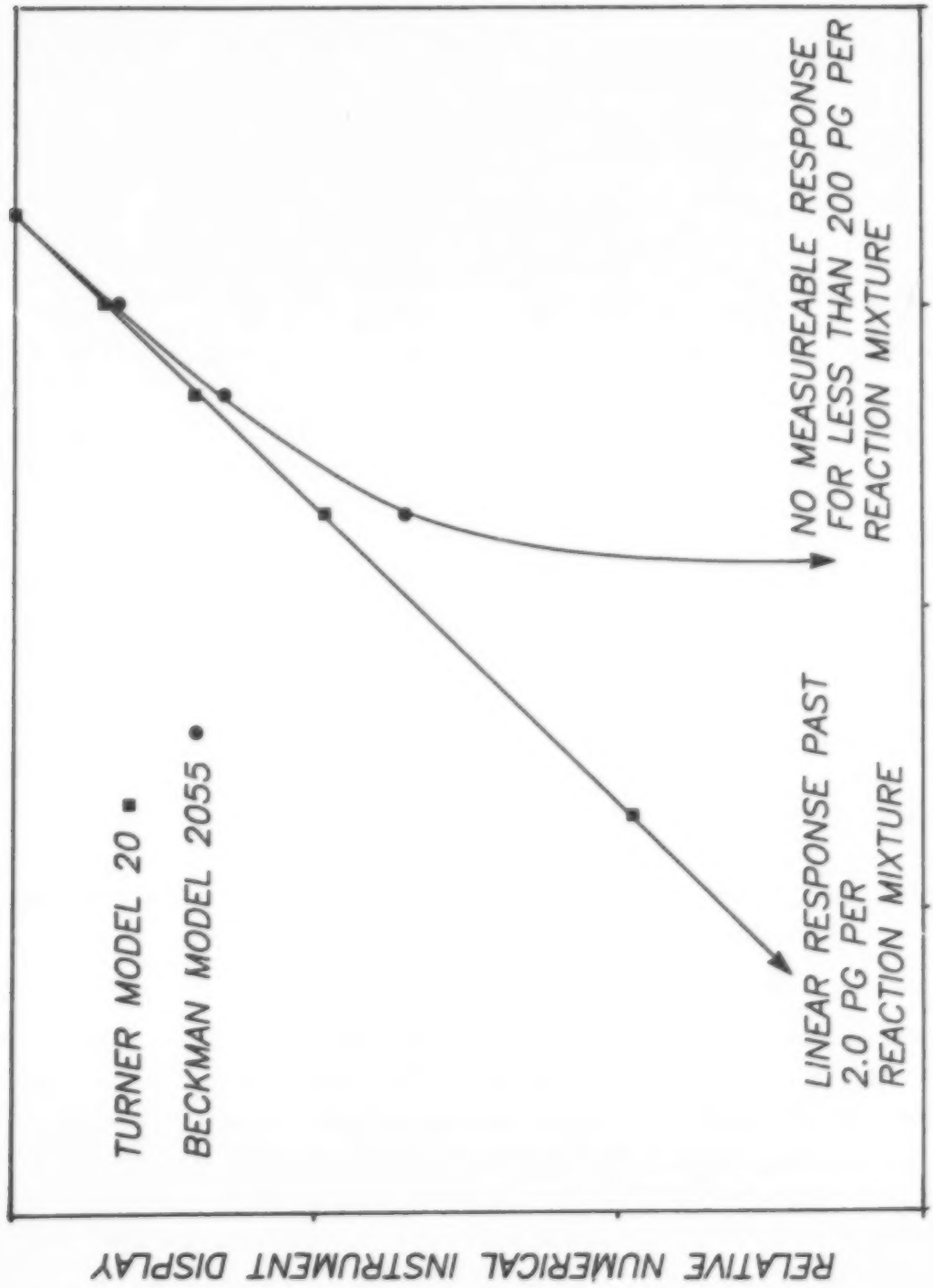

FIGURE 1 COMPARISON OF BECKMAN MICROTOX AND TURNER MODEL 20 PHOTOMETER

2.1 Temperature Control for Microtox™ Testing

Excellent temperature control for Microtox testing can be obtained in the Turner Designs Photometer by using heating/cooling recirculators such as the Lauda RM3-3S (Brinkman Instruments). The photochamber of the Turner Designs Photometer has a water jacket which can be connected to such a recirculator. To control the temperature of the Microtox reagent during incubation with a toxicant, an aluminum block cuvette holder can be constructed and immersed into a second recirculator.

Table 1 summarizes a comparison study of temperature control performances of the Beckman Microtox Analyzer and the Turner Designs Photometer run under laboratory conditions. Measurements were taken with a YSI thermocouple device which had been calibrated against an ASTM thermometer. The comparison was performed at 15.0°C, the temperature usually selected for Microtox testing. However, similar results were observed for the Turner-Lauda system at 25°C, but the Microtox Analyzer was not examined at this temperature. To obtain a temperature 15.0°C in the Turner photochamber, it was necessary to set the Lauda recirculator to 8.0°C. By setting the other Lauda recirculator to 15.0°C, and immersing the incubator block in the water of this recirculator, the desired temperature of 15.0°C in the Microtox cuvettes was obtained.

By examining Table I, it can be seen that by using the Lauda recirculators, the temperature control was equal, and in some aspects, superior to the Beckman Analyzer.

2.2 Instrument Response to Light from Luminescent Bacteria (Microtox™ Reagent)

In order to substitute for the Beckman Toxicity Analyzer, the relative response of the Turner Designs Instrument must relate well to the Beckman Instrument.

To determine how well the outputs of the two instruments correlate, two experiments were conducted.

In the first experiment, the sensitivity setting of both instruments were adjusted so that they would yield similar outputs. A cuvette containing Microtox reagents was placed in a well of the Analyzer incubator block which was set at 15°C. During incubation at this temperature, light from the reagent gradually decays and this decay was monitored by the two instruments. Measurements were made first in the Microtox Analyzer and then in the Turner Designs Photometer. Immediately following the measurements, the cuvette was returned to the incubator well. The average numerical output from the Beckman Analyzer was estimated from an attached chart recorder over a five to ten second period, while the output from the Turner Photometer was obtained

TABLE I COMPARISON OF TEMPERATURE CONTROL PERFORMANCE AT 15°C

	Beckman Model 2025 Toxicity Analyzer	Turner with Lauda Recirculators Model RM3-3T
1. Stability of photo-chamber temperature over five hours.	<0.1°C Change	<0.1°C Change
2. Stability of temperature in cuvette incubator over five hours.	<0.1°C Change	<0.1°C Change
3. Maximum variation in temperature between incubator wells.	0.1°C	<0.1°C
4. Maximum rise in temperature in cuvette from 0.2 ml to 1.5 ml level.	1.0°C	0.1°C
5. Maximum rise in temperature in cuvette from 0.2 ml to 2.0 ml level.	1.2°C	0.2°C

from the instrument's digital display produced when the delay time was set to 5 seconds and the integration time was also set to 5 seconds.

The results of this experiment which have been tabulated in Table II demonstrate an almost perfect correlation (correlation coefficient 0.9991) between the two instrument outputs.

A second experiment was conducted to determine whether this excellent correlation was maintained if the sensitivity setting of the instrument was changed. (Note: Since Microtox testing involves calculating a ratio of the light lost compared to the light remaining at a particular time and temperature, agreement of the absolute numerical value displayed by the two instruments is not required). The sensitivity settings were adjusted so that Turner Designs' numerical output was approximately 1/4 that of the Beckman Analyzer. Three cuvettes with different quantities of Microtox reagent were

TABLE II CORRELATION OF BECKMAN MICROTOX ANALYZER MODEL 2055 AND TURNER MODEL 20 RESPONSES TO LIGHT EMISSIONS FROM MICROTOX REAGENT:

Experiment I

Incubation Period at 15°C (Min)	Numberical Output of Instruments	
	Beckman	Turner Designs
0	95.8	86.1
7	89.4	81.2
14	84.5	77.9
24	79.7	73.1
35	74.2	68.3
45	67.0	63.3
50	67.2	61.9
59	60.7	57.3
70	55.2	52.2
83	50.0	46.8
94	44.7	42.5
106	40.4	38.4
120	35.7	33.9
180	10.0	9.0

CORRELATION COEFFICIENT = 0.9991

Experimental Protocol: Instrument Sensitivity settings were adjusted to yield similar outputs. Light decay from a cuvette containing Microtox Reagent was measured in the Beckman instrument, then in the Turner Designs.

incubated at 15°C for three time intervals (0,5, and 10 min.) and their light emissions were measured as in the previous experiment. Again, as shown in Table III, the two instrument outputs were almost perfectly correlated (correlation coefficient = 0.9997).

The above experiments indicate that the Turner Designs Model 20 Photometer in conjunction with controlled temperature recirculators can provide essentially the same function as the Beckman Microtox Toxicity Analyzer.

TABLE III CORRELATION OF BECKMAN MICROTOX ANALYZER MODEL 2055 WITH TURNER MODEL 20 RESPONSES TO LIGHT EMISSIONS FROM MICROTOX REAGENT:

Experiment II

Incubation Period at 15°C (Min)	Cuvette No.	Numerical Output of Instruments	
		Beckman	Turner Designs
0	No. 1	96.1	24.8
	No. 2	25.8	6.8
	No. 3	14.9	4.1
5	No. 1	94.6	23.8
	No. 2	22.9	5.8
	No. 3	14.7	4.1
10	No. 1	90.6	22.8
	No. 2	22.1	5.6
	No. 3	14.6	4.1

CORRELATION COEFFICIENT = 0.9997

Experimental Protocol: Instrument Sensitivity settings were adjusted so that the Turner Designs numerical output was approximately 1/4 that of the Beckman instrument. Three cuvettes with different quantitites of Microtox Reagent were then read on the instruments after three incubation periods. The light from each cuvette was measured first in the Beckman instrument, then in the Turner Designs.